

CHEMICAL ECOLOGY AND BIOLOGICAL CONTROL POTENTIAL
OF THE COCKROACH OOTHECAL PARASITOID
Aprostocetus hagenowii (Ratzeburg)

By

DANIEL ROBERT SUITER

A DISSERTATION PRESENTED TO THE GRADUATE SCHOOL
OF THE UNIVERSITY OF FLORIDA IN PARTIAL FULFILLMENT
OF THE REQUIREMENTS FOR THE DEGREE OF
DOCTOR OF PHILOSOPHY

UNIVERSITY OF FLORIDA

1994

UNIVERSITY OF FLORIDA LIBRARIES

ACKNOWLEDGEMENTS

Although one name appears on the title page of this dissertation, it would not have been possible without the resources and minds of several persons. Drs. P.G. Koehler and R.S. Patterson allowed me the latitude to pursue this nontraditional area of pest control, yet provided me with what I believe is a complete education. The cooperative research program these two scientists have built is extraordinary, and I feel fortunate to have been a part of their group for the past few years.

Special thanks go to Dr. Dave Carlson for allowing me to work out the chemical ecology of my project in his laboratory. Drs. Dave Carlson, Bob Vander Meer, and Jim Nation provided nearly 100 years of chemical ecology experience from which I benefitted greatly. Drs. Tom Walker and Richard Brenner provided insight on insect ecology, and Dr. A. I. Khuri and Mr. Jay Harrison provided helpful recommendations in statistical data analysis. Lloyd Davis helped me colonize the parasitoid. Behind every successful research project is a great technician; in my case it was Michelle Hosack. Simply, the chemical ecology aspect of my project would not have been possible without Michelle. I would like to thank my family for keeping the faith when others did not. Most of all, I would like to thank my dear friend and fiance, Lisa M. Ames, for her courage and patience. Advanced degrees are all about sacrifice, and we have sacrificed plenty over the past 5 years.

TABLE OF CONTENTS

	<u>Page</u>
ACKNOWLEDGEMENTS.....	ii
ABSTRACT.....	v
CHAPTER	
I. INTRODUCTORY STATEMENT.....	1
II. LITERATURE REVIEW.....	6
The Parasitoid.....	6
Kairomones.....	12
III. A SIMPLE REARING METHOD FOR THE COCKROACH OOTHECAL PARASITOID <u>Aprostocetus hagenowii</u> (Ratzeburg).....	17
Introduction.....	17
Materials and Methods.....	19
Results.....	24
Discussion.....	25
IV. SEASONAL INCIDENCE AND BIOLOGICAL CONTROL POTENTIAL OF <u>Aprostocetus hagenowii</u> (Ratzeburg) IN TREEHOLE MICROHABITATS.....	35
Introduction.....	35
Materials and Methods.....	37
Results.....	44
Discussion.....	46

V. A KAIROMONE FOR <u>Aprostocetus hagenowii</u> (Ratzeburg), A EULOPHID PARASITOID OF AMERICAN COCKROACH (<u>Periplaneta americana</u> (L.)) OOTHECAE.....	61
Introduction.....	61
Materials and Methods.....	63
Results.....	70
Discussion.....	74
REFERENCES CITED.....	87
BIOGRAPHICAL SKETCH.....	101

Abstract of Dissertation Presented to the Graduate School
of the University of Florida in Partial Fulfillment of the
Requirements for the Degree of Doctor of Philosophy

CHEMICAL ECOLOGY AND BIOLOGICAL CONTROL POTENTIAL OF THE
COCKROACH OOTHECAL PARASITOID Aprostocetus hagenowii (Ratzeburg)

By

Daniel Robert Suiter

August 1994

Chairman: Philip G. Koehler

Major Department: Entomology and Nematology

A simplified method of mass producing a female-biased colony of Aprostocetus hagenowii (Ratzeburg) on Periplaneta americana (L.) oothecae was developed. Forty-five percent of the 24 oothecae stung per day produced a clutch of parasitoids, 77% of which were female-biased clutches; six (range 0-18) female-biased clutches emerged per day. A significantly higher percentage of 0-1- (90%) and 2-3-wk-old (87%) oothecae produced a female-biased clutch than 4-5-wk-old (53%) oothecae. Development in 2-3- and 4-5-wk-old oothecae was significantly shorter, by 3 and 4 d, respectively, than development in 0-1-wk-old oothecae (35 d). The number of female (51) and male (7) progeny per clutch, and sex ratio (14% male) did not differ significantly among the three ages of oothecae.

Oothecae were irradiated or frozen to kill cockroaches before parasitism. A significantly higher percentage of irradiated-killed oothecae (100%) produced a female-

biased clutch than frozen-killed oothecae (47%), but per clutch, the number of female progeny (67) was not significantly different; irradiation dose did not significantly effect development of the parasitoid. Weekly for 30 wk, parasitoids were released in five cockroach-infested treeholes (20,000 females per treehole total). Parasitism of sentinel oothecae in release treeholes was 70-100% during release weeks 6 through 15, and 50%-80 during four additional weeks. Parasitism in control treeholes was 10-40%, but peaked at 46% the week of July 7; winter parasitism was <10%. Parasitism of oothecae in release treeholes remained higher for 10 of the 11 wk after parasitoid releases were terminated, when sentinel surveys were ended.

Chemically mediated host location was investigated in Y-tube bioassays. Nearly 80% of female parasitoids responded to an ootheca after 10 minutes. Bioassay of one ootheca equivalent from AgNO₃-impregnated silica gel identified the attractant as 6,9-heptacosadiene. This hydrocarbon is 37 times more abundant in frass than on the ootheca; it is the predominant hydrocarbon in frass, oothecae, and adult female cuticle. Its volatility from oothecae was demonstrated by trapping it on Super Q adsorbent, and its identity confirmed by comparison of its mass spectra with spectra from oothecal-6,9-heptacosadiene obtained by column chromatography. Coinjection of the volatile with 6,9-heptacosadiene resulted in a single GC peak at the retention time of 6,9-heptacosadiene.

CHAPTER I INTRODUCTORY STATEMENT

In recent years the structural pest control industry has undergone a rather significant change in pest control philosophy brought about, and paralleled by, the environmental movement in this country. Pest control professionals have experienced a strong increase in environmental legislation and regulation of their industry. These stepped-up pressures are a direct result of the public's increased awareness of the potential hazards of pesticide usage, especially in the urban environment where pesticides are purposefully applied to areas in direct contact with human inhabitants; by their very nature, application of pesticides in agroecosystems limits exposure to the general public. As a result of such regulatory pressure, the structural pest control industry is adopting less toxic, exposure-minimizing technologies for controlling urban pests. For example, the sale of child-safe bait stations to pest control companies has skyrocketed in the past decade, and more than doubled since 1986. The Age-of-Reduced-Exposure is also evidenced by an increase in the number of professional pest control companies now providing once a year, or as needed treatments.

The use of multiple control strategies to manage a pest population is often called integrated pest management (IPM), a term becoming more and more familiar to the structural pest control industry. IPM involves thorough inspection and diagnosis of the pest problem, followed by recommendation and implementation of strategies to manage

the pest population; management decisions are often based on population dynamics and biology of the pest. The use of toxic baits, inorganics, such as boric acid, and insect growth regulators, such as hydroprene, methoprene and fenoxycarb, are all alternatives to traditional pest control via application of organophosphate, carbamate, and pyrethroid residuals, and are gaining widespread acceptance for use in IPM programs for structural pests. Biological control of urban pests is yet another technique which warrants investigation as a component in IPM programs for certain urban pests. Because of the efficacious history of chemical pesticides, however, biological control has really never been given serious consideration, until quite recently.

There are several forms in which biological control can be achieved; the first is classical biological control. The target pest of a classical biological control program is usually an accidentally introduced species, as are most pest cockroach species in the U.S. When an organism is accidentally introduced, its primary parasites, predators and pathogens are often left behind in its native range. In its new environment the introduced species may near its biotic potential, limited mainly by abiotic factors and, in a few cases, newly-acquired natural enemies in its recently expanded range. The goal of classical biological control is simple; reacquaint the newly-introduced pest with natural enemies that held its numbers at equilibrium in its natural range. Classical biological control involves the exploration of a pest's native range for natural enemies that could be imported, quarantined, researched, and developed for biological control. Candidate material must be returned to the area of pest infestation, placed in quarantine and data gathered on its biology, pathogenicity, environmental impact, and effect on nontarget

organisms; data gathering of this magnitude is costly, and often requires many years.

Another way to achieve biological control is through the augmentation of natural enemies in the field by laboratory rearing and subsequent periodic release.

One problem arises with the concept of biological control in the urban environment. A difference between agricultural and urban pest control involves the concept of economic (agriculture) and aesthetic (urban) injury levels. In agriculture, a certain level of pest infestation is tolerated, and for biological control purposes is even desired. The level of the pest is held below the density which might cause economic loss. When the pest level reaches some threshold, action is taken to reduce its level. In urban entomology, where aesthetics, as opposed to crops or livestock, are being protected, the level of infestation tolerated is often one pest organism. Urban biological control is not being touted as a single control technique, but a technique to alleviate the reliance on pesticides.

Successful biological control programs for urban pests need to be efficacious and economically competitive with synthetic pesticides, or they will not survive in the marketplace. Market competitiveness will require that several criteria be met by the candidate product. Those criteria will include ease of application, efficacy, safety, production costs, and shelf life; biological control-based products must at least compete with sprayable pesticides on all these criteria. Several corporations have demonstrated the feasibility of developing biological control products, and have recently gone to market with them. Bio-Path, a bait station device, is loaded with a naturally occurring, soil-based pathogen that kills German cockroaches. Cockroaches crawl into the station, become

infected with fungal conidia, crawl away, become diseased, and die. This product has recently made its debut in the structural pest control market. The nematode-based product Vector was recently released for the outdoor application to areas infested with fleas.

BioSafe is a nematode based lawn and garden insect control product which is applied to the soil for control of cutworms, grubs, and other soil-dwelling insects. In each jug of BioSafe, 10 million nematodes are embedded in a gelatinous sheet and placed on a small piece of screen. Water is added, and after a 10 minute wait the product is ready to apply.

BioSafe has a shelf life of 6 months. The nematode used in BioSafe, Steinernema carpocapsae, has also been shown in field tests to infect and kill Australian and Smokybrown cockroaches in infested palm trees (D.R.S. unpublished).

The cockroach oothecal parasitoid Aprostocetus hagenowii (Ratzeburg) has some potential for the biological control of peridomestic cockroaches. A biological control program involving this parasitoid would combine periodic releases of it with a bait application to cockroach harborages. Baits will undoubtedly reduce cockroach populations, but unhatched oothecae, of which there may be hundreds, are unaffected by the bait and remain as a source of reinfestation. The use of biological controls for urban pests should be thought of as a preventative component in a multiple component integrated management system. A. hagenowii may help fill part of this niche, but a concerted effort must be put forth for its successful development.

With the continued regulation of the urban pest control industry, resulting in increased EPA registration revocations, it is inevitable that biological control practices will become useful tools in the integrated management of urban pests. Biological control

techniques will never replace chemical control techniques in urban pest management, but they have a future as a component in the integrated management of some urban pests.

Decades of liberal pesticide use has led to several hard lessons; environmental insult, endangerment of human health, and irreversible pesticide resistance leading to cycles of pesticide development are but a few. In the urban arena new pesticides (e.g. active ingredients) are not being developed. Development of a new active ingredient can often cost \$20 to \$40 million, so manufacturers are resorting to new formulations of the same actives. Biological control is safe, can be effective, and is often implemented for a fraction of the cost of new pesticide development.

CHAPTER II LITERATURE REVIEW

The Parasitoid

Aprostocetus hagenowii (Ratzeburg) (Hymenoptera: Eulophidae) is a small (2 mm), gregarious parasitoid of cockroach oothecae of the family Blattidae (Roth & Willis 1954, 1960, Cameron 1955, Edmunds 1955, Kanayama et al. 1975, Narasimham 1984, Kumarasinghe & Edirisinghe 1987, 1991). Host records include the oothecae of Periplaneta americana (L.), P. australasiae (F.), P. fuliginosa (Serville), P. japonica Karny, Blatta orientalis L., Eurycotis biolleyi Rehn, and E. floridana (Walker).

Distribution. The distribution of A. hagenowii is world-wide; its reported distribution is primarily from mild climates (10-30°N latitude), reflecting the distribution of its hosts. Cameron (1955) received parasitized oothecae from Trinidad, Saudi Arabia, and India, while Burks (1943) reported this parasitoid from the eastern and southern U.S., the West Indies, and the Hawaiian Islands. Ceianu (1986) reported A. hagenowii from B. orientalis in Romania (45°N latitude). Surveys of cockroach oothecal parasitoids in India uncovered A. hagenowii-parasitized P. americana, P. australasiae, and P. brunnea (Narasimham & Sankaran 1979, Kumarasinghe & Edirisinghe 1991). Vargas and Fallas (1974) discovered A. hagenowii parasitizing the oothecae of P. australasiae in San Jose, Costa Rica. Kanayama et al. (1975) and Takagi (1975) found A. hagenowii-parasitized P. fuliginosa oothecae in Japan.

Morphology/sexual differences. According to Edmunds (1955) and Ali and Islam (1983) male A. hagenowii are smaller than females and possess 9- or 10-segmented antennae which bear long setae; the antennae of females are 12-segmented, and do not bear long setae. Barlin et al. (1981) used scanning electron microscopy to document internal and external sensory structures on the antennae of male and female A. hagenowii. Among the many types of sensillae, a multiporous plate sensilla (types 1 and 2, organized in an alternating ring) in females seems the most likely candidate for host perception through chemoreception, probably olfaction with the abundance of pores, pore tubules, and dendrites in the antenna. Type 1 plate sensillae are more porous than type 2. Other types of sensillae found on the female antenna were (1) nonporous, socketed hairs, (2) multiporous, non-socketed hairs, and (3) multiporous pegs.

Mating. Mating in A. hagenowii occurred immediately after parasitoids emerged from the ootheca (Cameron 1955, Edmunds 1955, Vargas & Fallas 1974, Takahashi & Sugai 1982, Jie & Wenqing 1984). Takahashi and Sugai (1982) reported that males approached females after receiving sex pheromone, and exhibited a characteristic zig-zag walk with intermittent wing vibrations. Males exhibited violent wing-flapping and a quick, agitated gait while searching for females. Males then mounted females and began antennal stimulation; females responded to this by lifting their abdomen to initiate copulation, which took place following a backward movement of the male. Ether washes of female A. hagenowii elicited male zig-zag patterned movements.

Oviposition behavior. Cameron (1955) reported that oviposition began soon after mating was completed. Roth and Willis (1954), Edmunds (1955), Vargas and Fallas

(1974), and Narasimham (1984) described the oviposition behavior of A. hagenowii; females mounted and drummed the ootheca with their antennae, and occasionally stopped to groom their antennae or tap the ootheca with the tip of their abdomen (see Roth & Willis 1954, Plate 1A). After a suitable location on the ootheca was found, the female unsheathed her ovipositor and began to drill a hole through the ootheca wall. Edmunds (1955) reported that parasitoid eggs hatched in 24 h, but Narasimham (1984) reported an egg-hatching time of 48-54 h in P. americana oothecae. After oviposition, the female sometimes fed from the oviposition wound (Roth & Willis 1954, Plate 1D).

Narasimham (1984) studied the effect of superparasitism on developing broods. As the number of females allowed to concurrently oviposit into an ootheca increased, the number of emergent progeny per ootheca increased, as expected, but survival (i.e. number of emergent progeny divided by the estimated number of eggs deposited into an ootheca by a female) and developmental time decreased. In addition, the sex ratio increased (i.e. greater proportion of males). Emergent female parasitoids from superparasitized oothecae were also smaller, and their lifespan was shortened in comparison to female progeny from oothecae that were not superparasitized.

Although A. hagenowii parasitized oothecae of all ages, ca. 14-d-old oothecae were preferred. Roth and Willis (1954) demonstrated parasitism of P. americana oothecae up to 29-d-old, but not oothecae 35-d-old (i.e. just before hatch); there was some preference for 12 to 14-d-old oothecae. Narasimham (1984) demonstrated parasitoid preference for 11 to 15 d-old P. americana oothecae, but 30-d-old oothecae were also parasitized. Hagenbuch et al. (1988) exposed five ages (7-, 14-, 21-, 28-, and 35-d-old) of

P. americana oothecae to A. hagenowii; there were few differences in the proportion of oothecae parasitized, but development (i.e. oviposited egg to emergent adult) in 14-d-old oothecae was significantly shorter than in the four other ages. Kumarasinghe and Edirisinghe (1987) reported A. hagenowii oviposition in 0 to 34-d-old P. americana oothecae, but development only in 0 to 28-d-old oothecae.

Oviposition frequency. During her lifetime, a female will parasitize only one or two P. americana oothecae (Narasimham 1984, Hagenbuch et al. 1988, Heitmans et al. 1992). Larger females contained more eggs than smaller females. Large females distributed their eggs in two oothecae, but smaller females tended to parasitize only one ootheca (Heitmans et al. 1992). A majority of eggs were deposited during the first 2 d postemergence (Roth & Willis 1954). Jie and Wenqing (1984) reported peak parasitoid activity during the first 2-3 d postemergence.

Development. Developmental time (i.e. oviposited egg to emergent adult) of A. hagenowii is correlated (negative), as expected, with temperature; typical developmental time is ca. 30 days at 30°C. At 16-18°C, Cameron (1955) reported a developmental time of ca. 90 d in P. americana oothecae, but at 23°C development was completed in 24 d. Narasimham (1984) reported parasitoid development in P. americana oothecae of 61, 45.4, and 33.5 d at 15, 25, and 30°C, respectively. In mass-rearing, Hagenbuch et al. (1988) reported a developmental time in P. americana oothecae of 36 d at 27°C.

Cameron (1955), Edmunds (1955), and Narasimham (1984) provided detailed descriptions of immature (eggs and larvae) development (including sketches) in A. hagenowii. Eggs were banana-shaped, ca. 0.35 x 0.08 mm, and hatched in 24 or 48-54 h

(Edmunds 1955). There were 3 larval instars. The second and third instars lasted 4-5 and 10-22 d, respectively; the prepupal stage lasted 1-2 d, and at 18-21°C adult emergence occurred after ca. 10-22 d as exarate pupae. Roth and Willis (1954) provided close-up photographs of developing parasitoids within cockroach oothecae. Edmunds (1955) reported that larval feeding was completed in 18-25 d, and that there was an inverse relationship between brood size and developmental time. Edmunds (1955), Roth and Willis (1954), and Takagi (1975) demonstrated an inverse relationship between brood size and parasitoid size, probably due to limiting resources within the ootheca.

Sex ratio. The sex ratio of A. hagenowii is strongly female-biased. Roth and Willis (1954) and Cameron (1955) reported sex ratios of ca. 12-33 and 10% male in P. americana oothecae, respectively. Harlan and Kramer (1981) reported a sex ratio from P. americana and B. orientalis oothecae of 25% male, and Vargas and Fallas (1974) reported a sex ratio from P. australasiae oothecae of 38% male, and from E. biolleyi 32% male. In P. fuliginosa oothecae, sex ratios of 17 and 29% male were reported (Kanayama et al. 1975, Jie & Wenqing 1984). Sex ratio was influenced by host size and species (Narasimham 1984). Edmunds (1955) reported that unmated female parasitoids produced all-male egg clutches.

Rearing/diet/longevity. Unfed A. hagenowii adults typically live less than 10 d; adult lifespan can be extended, however, by provision of a carbohydrate diet to adult parasitoids. Male A. hagenowii provided raisins as a food source lived from 9-23 d (mean 15); females lived 19-45 d (mean 25). Unfed males lived 1.7 d, while unfed females lived 3.5 d (Edmunds 1955). Vargas and Fallas (1974) reported male and female parasitoid

survival of 6.33 (range 4-11) and 14.5 d (12-37 d), respectively. Parasitoids survived at 18°C with water and sugar for 2-6 wk (Cameron 1955). Jie and Wenqing (1984) reported adult longevities of 3-47 and 2-40 d for females and males, respectively.

Narasimham (1984) tested several diets (honey, flower mucilage, and sugar) for their ability to lengthen adult life. A honey diet was superior, as adult females and males lived a mean of 9 and 5 d, respectively, which was 6.2 and 3.5 d longer than starved individuals. Hagenbuch et al. (1988) described a rearing protocol for A. hagenowii and its host, P. americana oothecae. Parasitoids in their study were fed a honey diet, and lived 2-4 d longer than starved parasitoids. Without exception, in all studies, males lived a shorter time than females.

Parasitism in the field. Surveys of naturally-deposited oothecae suggest that A. hagenowii is of considerable importance in the natural control of Blattid cockroach pests. In Trinidad, Saudi Arabia, and India 15-57% of field-collected P. americana and P. australasiae oothecae contained A. hagenowii (Cameron 1955, Narasimham & Sankaran 1979). In another survey, parasitism by A. hagenowii in India revealed rates as high as 25 and 100% for P. americana and P. australasiae, respectively (Kumarasinghe & Edirisinghe 1991). In San Jose, Costa Rica, 33 and 52% of P. australasiae oothecae from two locations were parasitized by A. hagenowii (Vargas & Fallas 1974). In China, 0-93% of P. fuliginosa oothecae were parasitized by A. hagenowii (Jie & Wenqing 1984); parasitoid activity began in April, and peak field populations were found from May to August. In Japan, parasitism among oothecae collected from pig-pens and animal houses was between 49 and 62%; lab-reared oothecae placed in pig pens suffered ca. 35% parasitism

(Kanayama et al. 1975). In bird houses, 50% of P. americana and P. australasiae oothecae were parasitized (Hagenbuch et al. 1988).

Field tests. Purposeful release of A. hagenowii for control of Blattid cockroach species has had varying levels of success. Typically, researchers evaluate their release program by monitoring sentinel ootheca parasitism. Roth and Willis (1954) released A. hagenowii in rooms with sentinel P. americana oothecae distributed throughout. A release rate of 10 female parasitoids per sentinel ootheca resulted in ca. 75-85% parasitism. In experimental kitchens artificially infested with P. americana adults, Hagenbuch et al. (1989) released A. hagenowii and achieved 96% parasitism of viable oothecae after 7 wk. During the 8-11 wk period, parasitism rose to 98%, and at 12-17 wk was still above 95%. Parasitism of ceiling-deposited oothecae was significantly lower than in other areas. In their study, parasitoid release chambers accidentally allowed parasitoids to escape and enter control chambers holding P. americana oothecae; here parasitism was 46% after 7-11 wk, and 79.4% after 12-17 wk. Narasimham (1984) reported a preference of this parasitoid for warm, humid microclimates much like that of its hosts. In the same study, parasitoid activity was greatest at low humidities and high temperatures (35°C optimum).

Kairomones

Kairomones are interspecific semiochemicals (allelochemicals) which when produced, acquired by, or released as a result of the activities of an organism, which, when it contacts an individual of another species in the natural context, evokes in the receiver a behavioral or physiological reaction adaptively favorable to the receiver but not to the emitter (Nordlund & Lewis 1976). Vinson (1976, 1984a,b), Nordlund et al. (1981), and

Greany et al. (1984) provided excellent overviews of kairomone-dependent host selection by parasitoids, and list the following steps: (a) habitat location, including general habitat location, and potential host community (microhabitat) location, (b) host location, (c) host acceptance, (d) host suitability, and (e) host regulation. The "steps" involved in the host selection process are meant to be a framework only, and are only sometimes mutually exclusive; depending on the case, they may or may not all be present, and are often only vaguely separable. Host location and acceptance are the crux of the current research; the remainder of this review shall center on the abundance of primary literature involved in location and acceptance of hosts by parasitoids.

Host location, or finding, has been reviewed by several authors (Vinson 1976, Weseloh 1981), and is rather difficult to define. Weseloh (1981) defined chemical host location as the discovery, or perception, of a host from a distance due to short- (primarily) and long-range (rare) volatile compounds produced by the host, its activity, or one of its products. The cues responsible for discovery of the host can be physical (auditory, visual), chemical, or combinations of each.

Weseloh (1981) arbitrarily divided host location into long- (volatile) and short- (low volatility and tactile compounds) range chemoreception, and provided examples of parasitoids that locate hosts in these manners. Long-range cues serve to attract the parasitoid from a greater distance than short-range cues. Long-range host location cues are not as well-documented as short-range, relatively nonvolatile cues. A classic long-range host location cue used by parasitoids for host location is host sex pheromone (Nordlund et al. 1983, Lewis & Nordlund 1984, Noldus 1988, Noldus et al. 1991).

Although a wealth of literature involving the tritrophic interactions of a parasitoid and the long-range, volatile synomones produced by its host-plant complex exists, such interactions are not considered host location, but rather host microhabitat location, and will not be dealt with in this review. In short, parasitoids are often attracted to host microhabitats by volatile plant chemicals released as a result of herbivore damage (Elzen et al. 1983, 1984, 1986, Baehrecke et al. 1990, Keller 1990, Turlings et al. 1990, 1991a,b, McAuslane et al. 1991a,b).

Host location via detection of short-range kairomones has been well-documented and provides most of the host location literature. To utilize short-range kairomones as host location cues, parasitoids must come in close proximity to these low to nonvolatile compounds; the compounds must often be contacted. Commonly, these kairomones are found on or emanating from host-associated products or the host itself. Short-range kairomones commonly bring about a change in the behavior of the parasitoid when they are contacted, often releasing unique searching behaviors in the parasitoid that result in increased, intensive host seeking in a localized area.

After location of a host, the parasitoid will examine it, and either accept or reject it based on certain chemical and physical criteria. Arthur (1981) and Vinson (1976) reviewed chemical and physical stimuli utilized by parasitoids to examine hosts. Host examination leading to acceptance or rejection commonly involves antennal drumming behaviors mediated by nonvolatile compounds. Nonchemical examination cues leading to host acceptance or rejection include host size, shape, texture and color (Vinson 1976, Arthur 1981, Strand & Vinson 1983, Vinson & Piper 1986). It is generally thought that

acceptance/rejection is based on physical contact of the parasitoid with the host, thus chemical cues are almost always large, nonvolatile compounds.

In relation to the current research, there are a few host-related products of particular interest. Host frass is one of the most common materials utilized by parasitoids to locate hosts (Jones et al. 1971, Lewis & Jones 1971, Vinson et al. 1976, Henson et al. 1977, Nordlund & Sauls 1981, Weseloh 1981, Lewis & Nordlund 1984, Dmoch et al. 1985, Nordlund & Lewis 1985, Takabayashi et al. 1985, Van Leerdam et al. 1985, Clement et al. 1986, Drost et al. 1986, Nemoto et al. 1987a,b, Eller et al. 1988, Herard et al. 1988a,b, Lewis & Tumlinson 1988, Auger et al. 1989, Fukushima et al. 1989, Takabayashi & Takahashi 1989, McAuslane et al. 1990, Lewis et al. 1991, Ma et al. 1992). Both volatile and contact host location kairomones have been identified from host frass. Parasitoids characteristically antennate host frass and alter their search behavior when they contact the frass.

Chemical products of host glands (e.g. accessory, mandibular) are also often used by parasitoids to locate hosts (Vinson et al. 1975, Nordlund et al. 1977a,b, Mudd & Corbet 1982, Lewis & Nordlund 1984, Mudd et al. 1984, Van Driesche & Hulbert 1984, Muzaffar & Inayatullah 1986, Vinson & Piper 1986, Cave et al. 1987, Nordlund et al. 1987). Egg parasitoids, especially members of the Trichogrammatidae, are known to use host location kairomones from the egg hosts (Vinson 1975, Nordlund et al. 1977a,b, Leonard et al. 1987, Renou et al. 1989, Shu & Jones 1989). A variety of other host and host-related products have been reported to serve as host location and acceptance kairomones. Among them are honeydew (Vinson et al. 1978, Bouchard & Cloutier 1984,

1985, Hagvar & Hofsvang 1989), saliva (Jones et al. 1971, Lewis & Jones 1971, Clement et al. 1986, Ma et al. 1992), silk & cocoons (Dmoch et al. 1985, Weseloh 1987, Ma et al. 1992), larval trail pheromones (Howard & Flinn 1990), epideictic pheromones (Prokopy & Webster 1978), pupa/larva/exuvium/cocoon (Leonard et al. 1975, Vinson et al. 1976, Dmoch et al. 1985, Takabayashi et al. 1985, Takabayashi & Takahashi 1986, Herard et al. 1988a,b, Carde & Lee 1989, Takahashi et al. 1990, Ma et al. 1992), and wing scales (Lewis et al. 1972, Jones et al. 1973, Vinson 1975, Nordlund et al. 1976, Gueldner et al. 1984, Noldus & van Lenteren 1985a,b, Chiri & Legner 1986, Zaborski et al. 1987, Thomson & Stinner 1988, 1990, Shu & Jones 1989, Shu et al. 1990).

CHAPTER III
A SIMPLE REARING METHOD FOR THE COCKROACH OOTHECAL
PARASITOID Aprostocetus hagenowii (Ratzeburg)

Introduction

Aprostocetus hagenowii (Ratzeburg) is a small, gregarious, Eulophid parasitoid of oothecae from cockroaches in the family Blattidae. Its biology is well documented (Roth & Willis 1954, 1960, Cameron 1955, Edmunds 1955, Narasimham 1984). Host records include oothecae of these cockroaches: Periplaneta americana (L.) (American), P. australasiae (F.) (Australian), P. fuliginosa (Serville) (smokybrown), P. japonica Karny (Japanese), P. brunnea Burmeister (brown), Blatta orientalis L. (Oriental), Eurycotis floridana (Walker) (Florida woods), and E. biolleyi Rehn. A. hagenowii may prefer P. americana oothecae. Narasimham (1984) reported that A. hagenowii reared in P. australasiae oothecae for three generations subsequently preferred P. americana oothecae.

Female parasitoids emerge from the ootheca with their full complement of eggs, immediately sibmate, and can then parasitize a host; females typically deposited their eggs in one or two oothecae (Heitmans et al. 1992). Even though A. hagenowii will parasitize and develop in all ages of P. americana oothecae, up to ca. 28-d-old, 11 to 15-d-old oothecae were preferred (Narasimham 1984). Hagenbuch et al. (1988) exposed five ages (7-, 14-, 21-, 28-, and 35-d-old) of P. americana oothecae to A. hagenowii; there were

few differences in the proportion of oothecae parasitized, but those in 14-d-old oothecae emerged in a significantly shorter time.

Developmental time and number of emergent progeny per ootheca varies considerably. Narasimham (1984) reported parasitoid development, from oviposition to adult emergence, in P. americana oothecae, in 61, 45.4, and 33.5 d at 15, 25, and 30°C, respectively; Hagenbuch et al. (1988) reported a developmental time of 36 d at 27°C. Cameron (1955) reported 30-40 parasitoids from P. americana oothecae, and Harlan and Kramer (1981) reported ca. 30 parasitoids emerged from P. americana oothecae. In mass-rearing, P. americana oothecae produced ca. 56 parasitoids per ootheca (Hagenbuch et al. 1988).

The sex ratio of A. hagenowii is strongly female-biased. Cameron (1955) reported sex ratios of ca. 10 to 20% male, and Roth and Willis (1954) and Harlan and Kramer (1981) reported sex ratios of 11-33% and 25%, respectively. Edmunds (1955) reported that nonmated female parasitoids deposited all-male clutches. Narasimham (1984) reported that sex ratio was influenced by host size and species.

Narasimham (1984) and Edmunds (1955) studied the effect of superparasitism by A. hagenowii. As the number of females allowed to concurrently oviposit into an ootheca increased, the number of emergent progeny per ootheca increased, as expected, but survival (i.e. number of emergent progeny divided by the estimated number of eggs deposited into an ootheca by a female) and developmental time decreased; indeed, there was an inverse relationship between the number of emergent progeny and the developmental time. Sex ratio (i.e. proportion male) was also correlated (positive) with

the number of concurrently ovipositing females. Emergent female parasitoids from superparasitized oothecae were also smaller, and their lifespan was shortened in comparison to female progeny from oothecae that were not superparasitized.

It is not known whether this parasitoid takes carbohydrate in the field. However, carbohydrate diets (raisins, honey, sugar, flower mucilage) prolonged A. hagenowii's life by ca. 2-6 d (Cameron 1955, Edmunds 1955, Narasimham 1984, Hagenbuch et al. 1988); unfed parasitoids typically lived 2-4 d. A. hagenowii also fed from the sting wound after oviposition (Roth & Willis 1954, plate 1D). Because A. hagenowii was most active and deposited the majority of its eggs during the first 2-3 d post-emergence (Roth & Willis 1954, Jie & Wenqing 1984), the practicality of prolonging this parasitoids life with an artificial carbohydrate diet is questionable. The purpose of this study was to develop a large-scale, sex ratio-efficient (i.e. female-biased) rearing method for A. hagenowii.

Materials and Methods

Parasitoid. A. hagenowii was colonized in field-collected P. americana oothecae in the spring of 1993 from 10-20 adult female parasitoids collected at the Swine Research Unit on the University of Florida campus, Gainesville, Florida. A sample of parasitoids was sent to the U.S. National Museum in Washington, D.C., and identified as A. hagenowii by Dr. Mike Schauff. The colony was supplemented throughout the following year with parasitoids from sentinel P. americana oothecae placed in treehole microhabitats to monitor the natural population dynamic of A. hagenowii (Chapter IV).

Host production. P. americana oothecae were mass-reared in ootheca production units (OPU) according to the method of Hagenbuch et al. (1988). In short, the bottom of

each of eight galvanized steel tubs (59 liter) was removed and replaced with screen (0.65 by 0.65 cm mesh). The screened-bottom of each tub was then placed into an ootheca collection tray (43 by 43 by 9 cm); the floor of each tray was lined with brown construction paper onto which oothecae fell. The top rim of each tub had a 10-cm-wide inward projecting lip. The inner walls of the tub and the inside ceiling of the lip were coated with Teflon to keep cockroaches from escaping.

Cockroaches were obtained as needed from a *P. americana*-infested sewage treatment plant on the University of Florida campus (see Cornwell 1968 Fig. 115). One hundred to 300 mixed sex adult cockroaches were placed in each tub and provided water from a chick waterer, seven polyvinylchloride harborages (5 cm i.d. by 12 cm long), and laboratory rat chow (23% crude protein; Purina, #5001, St. Louis, MO). Dead cockroaches were replaced, water and food replenished, and oothecae collected weekly; time spent in colony maintenance was noted.

Parasitoid production. Daily, a cohort (some multiple of 6) of oothecae of varying, known age was placed individually into 18-ml snap cap vials (Thornton Plastics, Salt Lake City) each containing a single 2- or 3-d-old female parasitoid emerged from a known number of laboratory- and field-stung, sentinel oothecae (see Chapter IV). Cohorts were stored in plastic boxes (20 by 32 by 10 cm) and held at 27-30°C, in ambient light and humidity. Each d for 45 d, the number of female- and male-biased parasitoid clutches which emerged were recorded for each cohort, and summed for all cohorts. After 45 d, the number of oothecae producing anything other than parasitoids (i.e. (1) cockroaches or (2) neither parasitoids nor cockroaches) were recorded for each cohort. All vials

containing newly emerged progeny were placed daily, with snap caps intact, into a Plexiglass cage (30 by 30 by 30 cm; Hagenbuch et al. 1988) and held for 24 h to ensure sibmating. After 24 h the snap cap was removed from each vial, allowing parasitoids to mix. Water and food were provided from water and honey-water soaked cotton balls placed individually into souffle cups. Parasitoids were held for an additional 2 d, and then killed by holding the cage at 4°C for 24 h.

Parasitoid development in aged oothecae. The development of *A. hagenowii* in 1 to 8-, 15 to 21-, and 30 to 36-d-old (fixed qualitative factor, 3 levels) pristine (i.e. not dented, and without attached detritus) *P. americana* oothecae was investigated in a randomized complete block design (RCBD) with seven clutches (extraneous factor) of parasitoids serving as blocks; parasitoids from each clutch were the progeny of a single female. A RCBD was used because females within a clutch (i.e. sisters) are more similar genetically than females between clutches; interclutch genetic variability among females was assumed beforehand to contribute to variability in response variables.

For each clutch, ten oothecae of each age were randomly selected and placed individually into 18-ml snap cap vials containing a single, randomly selected 2-d-old, unfed female parasitoid. Oothecae were incubated at 30°C under natural lighting until cockroaches or parasitoids emerged, at which time cockroaches were discarded, but parasitoids were stored in 70% isopropyl alcohol until they could be sexed and counted. Response variables from oothecae that produced a female-biased clutch of parasitoids were (1) number of emergent female progeny per clutch, (2) number of emergent male progeny per clutch, and (3) developmental time, defined as the time (d) from experiment

initiation until the day of progeny emergence. If neither cockroaches nor parasitoids had emerged after 45 d, oothecae were counted and discarded. For each age, across all clutches, was recorded the fate of each ootheca. Each ootheca could have produced (1) a female-biased clutch of parasitoids, or (2) a male-biased clutch of parasitoids, or (3) cockroaches, or (4) neither parasitoids nor cockroaches.

Descriptive statistics. Data for parasitoid and host colonies were summarized with the descriptive statistics mean, standard deviation, mode, highest value, 90, 75, 50 (=median), and 25th percentiles, and the lowest value. A percentile is the percentage of values in the observed data set which are lower than the data value reported.

Categorical data analysis. Differences in proportions among the three ages of oothecae (rows) for each of the four responses (columns), ((1) number of oothecae from which emerged a female-biased clutch, or (2) number from which emerged a male-biased clutch, or (3) number from which emerged cockroaches, or (4) number from which emerged neither parasitoids nor cockroaches) were analyzed in a 3 by 4 contingency table with a Chi-Square Homogeneity of Proportions test (Ott 1988, p. 253) since row marginal totals were fixed ($n=70$) in advance. Proportions among ootheca ages (i.e. within each column) were separated by noninclusion of zero in a 95% confidence interval constructed on the difference in proportions between oothecal ages (i.e. pairwise comparisons of ootheca age proportions within each column) (Hochberg & Tamhane 1987, p. 275 for formula).

Equal variance assumption and analysis of variance. The assumption of equality of variances (H_0 : Variances are equal versus H_A : At least two variances are unequal) among

oothecal ages in the RCBD experiment was tested with Levene's test (Levene 1960, Conover et al. 1981) for the following six response variables: (1) number of female progeny per clutch, (2) number of male progeny per clutch, (3) the arcsine of the square root of the sex ratio, (4) developmental time, (5) the square root of developmental time, and (6) the log of developmental time. In Levene's test, the mean of each sample is subtracted from each data point within the sample, and an analysis of variance (PROC GLM in SAS) conducted on the absolute values of these differences (SAS Institute 1988). In the RCBD experiment, means were computed for each ootheca age across all clutches. The corresponding SAS MODEL for Levene's test was $Y = \text{age}$. The prechosen alpha level of the test was 0.10. Levene's test indicated non-significantly different variances among ootheca ages for the number of female ($F=0.00$, $df=2,161$, $P=0.9986$) and male ($F=0.99$, $df=2,161$, $P=.3746$) progeny per clutch, and the arcsine of the square root of the sex ratio ($F=1.05$, $df=2,161$, $P=.3517$). Subsequently, these variables were analyzed by analysis of variance with the corresponding SAS MODEL for a RCBD, $Y = \text{age clutch}$; both hypotheses were tested with the mean square for error at $\alpha=.05$.

Nonparametric analysis. Developmental time ($F=15.28$, $df=2,161$, $P=.0001$), the square root of developmental time ($F=15.19$, $df=2,161$, $P=.0001$), and the log of developmental time ($F=14.65$, $df=2,161$, $P=.0001$) grossly violated the assumption of equal variance. Therefore, developmental time was alternatively analyzed with a Friedman-type test (Mack & Skillings 1980, Skillings & Mack 1981); in this test, a Kruskal-Wallis procedure (PROC NPAR1WAY in SAS) is conducted on each block, yielding a X^2 and degrees of freedom. The X^2 test statistic and degrees of freedom are summed over all

blocks and compared to a critical X^2 value at the summed degrees of freedom and prechosen alpha level (.05) of the test. Median developmental times were separated by pairwise comparisons using the same technique at $\alpha=.01$.

Results

Approximately 24, 2-wk-old oothecae were stung per d (Table 3.1). Although the largest number of oothecae stung per d was 72, the interquartile range (i.e. 75th percentile-25th percentile) was 6 oothecae. Likewise, the oldest oothecae stung were ca. 34-d-old, but the interquartile range was ca. 10 d. Daily cohorts of oothecae were stung from 2- and 3-d-old female parasitoids that had hatched from ca. 12 laboratory-reared oothecae plus a few field-stung oothecae; parasitoids from field-stung oothecae were not available daily, as indicated by a mode of zero, but the colony was infused with field genes whenever possible.

From the 176 daily cohorts of oothecae from which data were gathered, 54.9% of them either produced cockroaches or nothing; female-biased clutches accounted for 77% of the oothecae which produced a clutch of parasitoids (Table 3.2). Production of male-biased clutches, the result of nonmated females, was ca. 10% from each cohort, while ca. 20% of oothecae in each cohort hatched neither parasitoids nor cockroaches.

For all cohorts, there were nearly 6 (mean=5.6) female-biased clutches produced per d compared to only ca. 2 male-biased clutches; the largest number of female-biased clutches produced per d was 18, but the most common (=mode) was 5. About 30 minutes per d was obligated to parasitoid colony maintenance (Table 3.3).

Weekly, several hundred (mean=464, median=410) *P. americana* oothecae were harvested for parasitoid colony maintenance, and laboratory and field experiments; the largest number harvested in a wk was 1,070 (Table 3.3). Typical host colony working time was 2-3 h, once a wk.

In the RCBD experiment, a significantly greater percentage of 1 to 8- and 15 to 21-d-old oothecae produced a female-biased clutch than the 30 to 36-d-old oothecae (Fig. 3.1). This was a result of *A. hagenowii*'s reduced ability to develop in 30 to 36-d-old oothecae, as evidenced by a significantly greater percentage of 30 to 36-d-old oothecae producing cockroaches than the younger oothecae.

Analysis of variance among the oothecal ages indicated no difference in the number of females ($P=.0997$), males ($P=.5850$), and sex ratio ($P=.3405$) per clutch (Tables 3.4 and 3.5). By pooling all data ($n=164$ clutches), the mean (95% confidence interval) number of female and male progeny per clutch, and sex ratio, was 51.1 females (48.7-53.5), 9.0 males (7.5-10.4), and .139 male (.121-.158), respectively. Parasitoid developmental time, however, differed significantly among the 3 ages of oothecae ($P<.005$); developmental in 15 to 21- and 30 to 36-d-old oothecae was 3 and 4 d shorter, respectively, than developmental time in 1 to 8-d-old oothecae (Table 3.5).

Discussion

The only published attempt at large scale production of *A. hagenowii* was by Hagenbuch et al. (1988). One of the most important factors limiting large scale production of this parasitoid is production of host oothecae. Hagenbuch et al. (1988) produced ca. 200-250 *P. americana* oothecae per wk; because of the accessibility of adult cockroaches

in the present study, weekly oothecal production averaged 464 (range 51-1,070) (Table 3.3). Indeed, oothecal production in this study was limited by colony space, rather than numbers of cockroaches.

Research on artificial diets for rearing larval parasitoids is greatly needed. Because of the physical, visual and chemical complexity in the host selection process by parasitoids (Vinson 1976), as well as the physiological and immunological constraints within the host after oviposition, studies on artificial diets necessarily involve a thorough understanding of the chemical nature of the host selection process. Of primary importance is the identification of internal and external host acceptance and oviposition stimulants (Li 1990), as well as nutritional balance in the artificial diet. Because of these constraints, it is doubtful that an artificial diet for this parasitoid will be developed in the near future.

The reproductive strategy of *A. hagenowii* is such that presentation of a single foundress with a single host results in a strongly female-biased sex ratio (Table 3.5). In this study, production of female-biased clutches was 5.6 per d (Table 3.3), or 1,900-2,100 females per wk from ca. 168 oothecae. Additionally, ca. 86.1% of emerging parasitoids were female. Hagenbuch et al. produced ca. 6,160 adult parasitoids per wk from ca. 196 oothecae, but due to their rearing procedure, where superparasitism was common, only 53% were female.

In superparasitism, the number of progeny per ootheca and sex ratio increases as the number of foundresses per ootheca increases (Edmunds 1955, Narasimham 1984), resulting in more parasitoids per clutch, but a higher proportion of males. Heitmans et al. (1992) and Narasimham (1984) have also shown that the size (mm) and longevity of

female progeny decreases with clutch size. Daughters from a single foundress are larger, and live longer, than the female progeny from superparasitism. Also, egg supply in females is strongly correlated (positive) with body size. Large females often distribute their eggs in two hosts, with the second clutch always smaller and with a smaller probability of survival than the first; small females typically parasitize a single host. A single, large parasitoid will not deposit all her eggs in one host when only one host is available (Heitmans et al. 1992). If two hosts are available she will parasitize both, but the first clutch will be larger than the second. Collectively, the progeny from parasitism of two oothecae is greater than parasitism of only a single ootheca.

Several problems with parasitoid rearing involving superparasitism are evident from the viewpoint of a practical biological control program. First, superparasitism results in an inordinate number of useless male progeny (47% male) and fewer female progeny than the single foundress method (13.9% male). Second, female progeny from superparasitism do not live as long as females from a single foundress. This has implications for augmentative field release, because small females cannot search for hosts for as long as larger females. And last, small females have fewer eggs and typically only parasitize one host, while larger females parasitize two hosts.

The literature reporting general biological parameters (e.g. clutch size, sex ratio, developmental time) of A. hagenowii has rarely (excluding Heitmans et al. 1992) taken into consideration interclutch genetic variability among females when using them as mothers for stinging experiments. In the present study, the RCBD experiment was used to eliminate that extraneous source of variation. In fact, the analysis of variance indicated a

significant clutch effect for the number of female ($P=.0001$) and male ($P=.0001$) progeny per clutch, and the sex ratio ($P=.0001$) (Table 3.4). In general, the results of this study (Table 3.5 and Fig. 3.1) agree quite well with those of other researchers (Roth & Willis 1954, Cameron 1955, Harlan & Kramer 1981, Narasimham 1984, Hagenbuch et al. 1988).

A. hagenowii is a parasitoid that has potential as a tool in the integrated management of peridomestic cockroaches. In uninhabited yet sensitive environments, augmentative releases of the parasitoid coupled with the use of toxic baits might eradicate existing populations of cockroaches. However, such a program would require a scheduled, dependable, and large supply of oothecal parasitoids. The present study has shown that a sex ratio-efficient (i.e. female-biased) colony of this parasitoid can be maintained.

Table 3.1. Descriptive statistics of parasitoid colony maintenance.

Variable	Mean ^a	Std. Dev.	Mode	Highest value	90th percentile	75th percentile	Median	25th percentile	Lowest value
# Ooethecae/ cohort	26.5	8.4	24	72	36	30	24	24	6
Ooethecal age (d)	15.1	6.5	19	34	22.5	19.5	16	9.8	3.5
Colony ooethecae ^b	12.2	7.9	11	42	23	16	11	6	0
Field ooethecae ^b	4.3	4.0	0	20	9	6.5	3	1	0

^a All descriptive statistics based on n=176 d of colony maintenance.

^b The number of colony (field) ooethecae from which 2 to 3-d-old female parasitoids were randomly drawn to sting the cohort.

Table 3.2. Descriptive statistics of the results from parasitized cohorts of oothecae.

Variable (%)	Mean ^a	Std. Dev.	Mode	Highest value	90th percentile	75th percentile	Median	25th percentile	Lowest value
Female-biased clutches	34.5	20.2	33.3	91.7	62.5	50	33.3	16.7	0
Male-biased clutches	10.6	9.8	0	50	25	16.7	8.3	4.2	0
Cockroaches	34.9	20.0	33.3	100	61.1	47.9	33.3	20.4	0
Neither	20.0	13.3	12.5	66.7	38.9	25.8	16.7	12.5	0

^a Descriptive statistics based on n=176 cohorts of oothecae; mean=26.5, mode & median=24 oothecae/cohort (see Table 3.1).

Table 3.3. Descriptive statistics of production from and time allocation for parasitoid and host colonies.

Variable	n ^a	Mean	Std. Dev.	Mode	Highest value	90th percentile	75th percentile	Median	25th percentile	Lowest value
<u>PARASITOID COLONY</u>										
Female-biased clutches/d	310	5.6	3.3	5	18	10	7	5	3	0
Male-biased clutches/d	310	1.8	1.6	1	10	4	2	1	1	0
Labor (min/d)	241	31.3	10.1	30	65	45	40	30	25	10
<u>HOST COLONY</u>										
# Harvested/wk	57	464	264	51	1,070	790	668	410	237	51
Labor (h/wk)	38	2.4	0.5	2	3.5	3.1	2.8	2.5	2	1.3

^a For PARASITOID COLONY, n is number of replicate days; data covers from May 8, 1993 to March 13, 1994. For HOST COLONY, n is number of replicate weeks; data covers from January 18, 1993 to March 13, 1994.

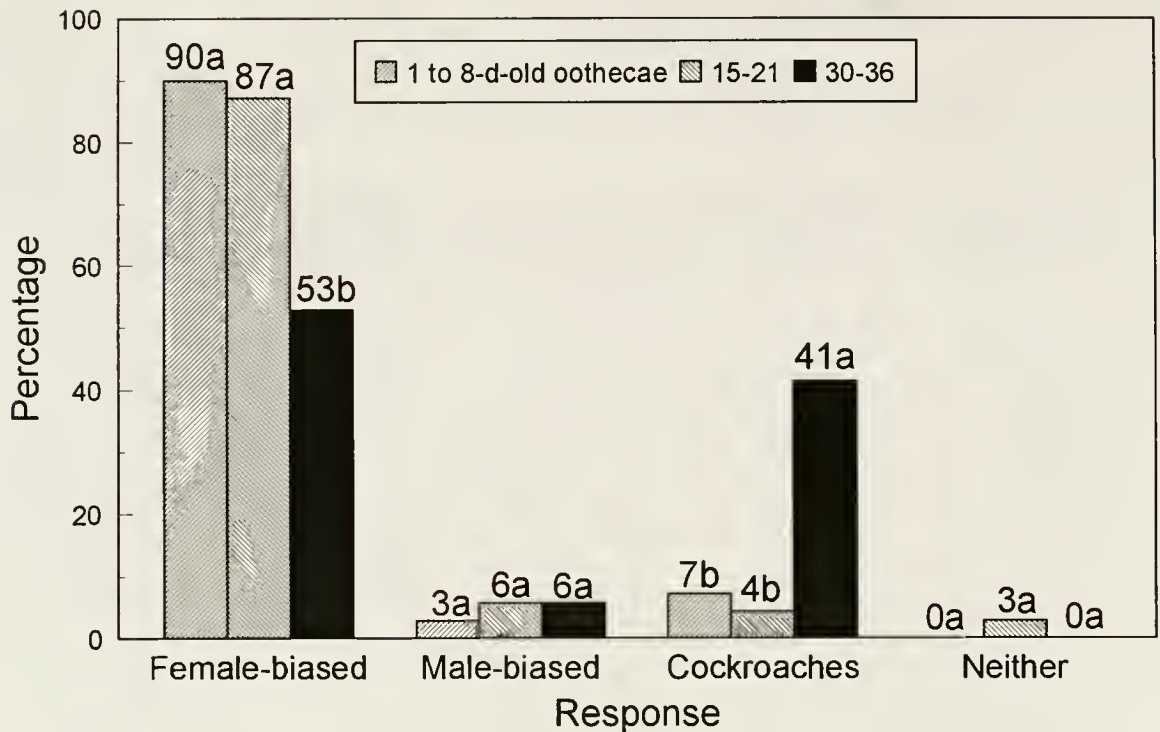


Fig. 3.1. Percentage of oothecae from which emerged (1) a female-biased clutch of parasitoids, or (2) a male-biased clutch of parasitoids, or (3) cockroaches, or (4) neither parasitoids nor cockroaches in the RCBD experiment. Chi-square Homogeneity of Proportions test (Ott 1988, p. 253): $X^2=46.5$, $df=6$, $P<.001$. For each response, bars separated by different letters are significantly different. Proportions within each response were separated by non-inclusion of zero in a 95% confidence interval constructed on the difference in proportions between ootheca ages (i.e. pairwise comparisons of age proportions within each response) (Hochberg & Tamhane 1987, p. 275 for formula).

Table 3.4. Analysis of variance for the number of female and male progeny per clutch, and the arcsine of the square root of sex ratio (RCBD experiment).

Dependent Variable	Source ^a	Sum of Squares	Mean Square	F Value	P Value
# Females	Age	970.537	485.269	2.34	0.0997
	Clutch	6645.085	1107.514	5.34	0.0001
	Error	32144.152	207.382		
	Total	39759.774			
# Males	Age	69.557	34.779	0.54	0.5850
	Clutch	4674.659	779.110	12.05	0.0001
	Error	10020.729	64.650		
	Total	14764.945			
Arcsine	Age	0.03921	0.01960	1.08	0.3405
	Clutch	1.27035	0.21173	11.72	0.0001
	Error	2.80085	0.01807		
	Total	4.11041			

^aFor each analysis of variance, the degrees of freedom for Age, Clutch, Error, and Total are 2, 6, 155, and 163, respectively.

Table 3.5 Number of female and male *A. hagenowii* per clutch, sex ratio, and developmental time in 3 ages of *P. americana* oothecae (RCBD experiment).

Age (d)	n	Response Variable ^a			Developmental time/clutch (d) ^c (median)
		Number females/ clutch ^b (mean±SE)	Number males/ clutch ^b (mean±SE)	Sex ratio/clutch ^b (mean & 95% CI)	
1-8	64	52.5 a ± 2.0	9.3 a ± 1.2	.143 a (.116-.173)	35 a
15-21	62	52.5 a ± 1.9	8.2 a ± 1.0	.127 a (.104-.151)	32 b
30-36	38	46.6 a ± 2.4	9.7 a ± 2.0	.155 a (.108-.210)	31 b

^a Means or medians within a column followed by the same letter are not significantly different.

^b See Table 3.4 for analysis of variance.

^c Modified Friedman's test (Mack & Skillings 1980, Skillings & Mack 1981) on developmental time; $X^2=82.9$, $df=14$, $P<0.005$. Medians separated by the same technique by pairwise comparison ($\alpha=.01$) of developmental times.

CHAPTER IV
SEASONAL INCIDENCE AND BIOLOGICAL CONTROL
POTENTIAL OF Aprostocetus hagenowii (Ratzeburg)
IN TREEHOLE MICROHABITATS

Introduction

The Hymenopteran natural enemies of cockroaches were recently reviewed (LeBeck 1991), and several show potential as candidates for biological control. One of those natural enemies, Aprostocetus hagenowii (Ratzeburg), is a small, gregarious Eulophid parasitoid of cockroach oothecae of the Blattidae (Roth & Willis 1954, Cameron 1955, Edmunds 1955). Piper et al. (1978) surveyed Hymenopteran oothecal parasitoids in several Texas and Louisiana cities inhabited by Periplaneta americana (L.) (American cockroach), P. fuliginosa (Serville) (smokybrown cockroach), P. brunnea Burmeister (brown cockroach), and Blatta orientalis L. (Oriental cockroach). About 25% of the 2,712 oothecae collected were parasitized by A. hagenowii and Evania appendigaster (L.); 99.4% of parasitized oothecae were parasitized by A. hagenowii. This parasitoid was present at 14 of the 17 Texas and all 4 Louisiana sites. P. americana oothecae suffered the highest rate of parasitism (46%), even though they accounted for only 8.6% of the oothecae sampled. P. fuliginosa made up 87% of the oothecae sampled, but suffered ca. half the rate of parasitism of P. americana oothecae.

Fleet and Frankie (1975) surveyed rates of P. fuliginosa ootheca parasitism at several cockroach-infested homes in Texas; rates of ootheca parasitization were 22.2 to

84.2% for oothecae collected in the outdoor urban environment. All surveys conducted in their study were in the summer or fall of the year.

Several authors investigated the effect of indoor releases of A. hagenowii on the rate of parasitization of sentinel oothecae. Roth and Willis (1954) obtained 83% parasitism of sentinel oothecae in a room (5 m by 5 m by 4 m high) when parasitoids were released at a rate of 10 females per sentinel ootheca. Hagenbuch et al. (1989) released ca. 600 parasitoids per wk in experimental chambers (2.4 m by 2.4 m by 2.8 m high) artificially infested with adult P. americana; after 7 wk, parasitism of naturally-deposited oothecae was >95%. The resulting sex ratio was 1 male to 0.71 females, indicating severe superparasitism when the release rate was 8.3 female parasitoids per host. In indoor residences, Piper and Frankie (1978) attained maximum rates of P. americana ootheca parasitization of 57-62% with releases of 8 and 12 female parasitoids per ootheca. In plumbing chases, Pawson and Gold (1993) obtained a parasitism rate of 30.6% (range 23-39%) of sentinel P. americana oothecae with as few as 300 (275-415) female parasitoids released per wk from parasitized P. americana oothecae; 300 (229-355) parasitoids released every 2 wk resulted in ca. 18.2% sentinel ootheca parasitism (range 2-44%). A. hagenowii found sentinel oothecae placed in all locations within the plumbing chase; sentinel oothecae placed 16 m from the release site were parasitized.

The four studies described above indicate that A. hagenowii indeed has some potential as a biological control organism of peridomestic cockroaches. However, none of these studies investigated the effect of an outdoor release of A. hagenowii on sentinel oothecae. Primary peridomestic cockroach foci are located outdoors in visually

identifiable, limited harborages such as trash piles, under wooden decks, and in trees and treeholes (Fleet et al. 1978, Appel & Rust 1985, Brenner 1988, Brenner & Pierce 1991). The release of oothecal parasitoids in these habitats seems most logical. The objective of this study was to evaluate the potential of prolonged, inundative release of A. hagenowii for biological control of peridomestic cockroaches in treehole microhabitats.

Materials and Methods

Parasitoid development in irradiated and frozen oothecae. A disadvantage of releasing parasitoids from laboratory-parasitized oothecae is the likelihood of further, unintentional cockroach infestation of the habitat. About 35% of the time (see Table 3.2) cockroaches emerge from an ootheca placed in a vial with a female parasitoid. Therefore, the development of A. hagenowii in 13 to 19-d-old irradiated-killed, frozen-killed, and unkilld (fixed qualitative factor, 3 levels) P. americana oothecae was investigated in a randomized complete block design (RCBD) with three clutches (extraneous factor) of parasitoids serving as blocks; parasitoids from each clutch were the progeny of a single female. A RCBD was used because females within a clutch (i.e. sisters) are more similar genetically than females between clutches; interclutch genetic variability among females was assumed beforehand to contribute to variability in response variables.

Oothecae were treated by freezing (-16°C) for 24 h, or irradiating (2.5 kR) with a Cesium-137 irradiator (Radiation Machinery Corporation, Parsippany, NJ); control oothecae were not treated. To monitor cockroach mortality from treatment, an additional group of oothecae (n=15 for both treatments and control) were treated the same but not parasitized. For each clutch, within 2 h of removal from the freezer or post-irradiation five

oothecae from each treatment and the control were randomly selected and placed individually into 18-ml snap cap vials containing a single, randomly selected 2-d-old, unfed female parasitoid. Oothecae were incubated at 30°C under natural lighting until cockroaches or parasitoids emerged, at which time cockroaches were discarded, but parasitoids were stored in 70% isopropyl alcohol until they could be sexed and counted. Response variables from oothecae that produced a female-biased clutch of parasitoids were (1) number of emergent female progeny per clutch, (2) number of emergent male progeny per clutch, and (3) developmental time, defined as the time (d) from experiment initiation until the day of progeny emergence. If neither cockroaches nor parasitoids had emerged after 45 d, oothecae were counted and discarded. For each treatment, across all clutches, was recorded the fate of each ootheca. Each ootheca could have produced (1) a female-biased clutch of parasitoids, or (2) a male-biased clutch of parasitoids, or (3) cockroaches, or (4) neither parasitoids nor cockroaches.

In a second experiment, the effect of ootheca age (hereafter age) and irradiation dose (hereafter dose) on the production of *A. hagenowii* was investigated in a 3 by 4 factorial experiment with blocking employed (see Ott 1988, p. 696 for design); 8 clutches (hereafter clutch) served as blocks. The fixed qualitative factor age had 3 levels, 3 to 11-, 12 to 18-, and 19 to 25-d-old, while the fixed factor dose had 4 quantitative levels, 2.5, 5, 20, and 40 kR of radiation. For each clutch, 2 oothecae from each age/dose combination were randomly selected and placed individually into 18-ml snap cap vials containing a single, 2-d-old, unfed female parasitoid. The total number of oothecae in the factorial experiment was 3 (ages) x 4 (doses) x 8 (clutches) x 2 (replicates)= 192. Response variables were identical to those described for the RCBD experiment.

Categorical data analysis. For the RCBD experiment, differences in proportions among the three treatments (rows) for each of the four responses (columns) ((1) number of oothecae from which emerged a female-biased clutch, or (2) number from which emerged a male-biased clutch, or (3) number from which emerged cockroaches, or (4) number from which emerged neither parasitoids nor cockroaches) were analyzed in a 3 by 4 contingency table with a Chi-square Homogeneity of Proportions test (Ott 1988, p.253) since row marginal totals were fixed ($n=15$) in advance. Proportions among the treatments (i.e. within each column) were separated by non-inclusion of zero in a 95% confidence interval constructed on the difference in proportions between treatments (i.e. pairwise comparisons of treatment proportions within each column) (Hochberg & Tamhane 1987, p. 275 for formula).

Equal variance assumption testing and analysis of variance. The assumption of equality of variances (H_0 : Variances are equal versus H_A : At least two variances are unequal) among treatments in the RCBD experiment was tested with Levene's test (Levene 1960, Conover et al. 1981) for the following four response variables: (1) number of female progeny per clutch, (2) number of male progeny per clutch, (3) the arcsine of the square root of the sex ratio, and (4) developmental time. In Levene's test, the mean of each sample is subtracted from each data point within the sample, and an analysis of variance (PROC GLM in SAS) conducted on the absolute values of these differences (SAS Institute 1988). In the RCBD experiment, means were computed for each treatment across all clutches. The corresponding SAS MODEL for Levene's test was $Y = \text{treatment}$. The prechosen alpha level of the test was 0.10. Levene's test indicated non-significantly

different variances among treatments for the number of female ($F=0.14$, $df=2,32$, $P=.8656$) and male ($F=0.73$, $df=2,32$, $P=.4906$) progeny per clutch, arcsine of the square root of the sex ratio ($F=0.20$, $df=2,32$, $P=.8214$), and developmental time ($F=0.86$, $df=2,32$, $P=.4325$). Subsequently, these variables were analyzed by analysis of variance with the corresponding SAS MODEL for a RCBD, $Y = \text{treatment clutch}$; both hypotheses were tested with the mean square for error at $\alpha=.05$

For the factorial design, Levene's test was conducted on the means of each factor level combination, across all clutches, by analysis of the main effects and their interaction; the SAS MODEL statement for Levene's test was $Y = \text{dose age dose*age}$. The number of females ($F=1.02$, $df=11,113$, $P(\text{total model})=.4356$) and the log of the number of males ($F=1.16$, $df=11,116$, $P(\text{total model})=.3213$) were found to have variances that did not differ significantly, and were therefore analyzed by analysis of variance with the SAS MODEL $Y = \text{dose age dose*age clutch}$ (see Ott 1988, p.696 for analysis).

Parasitoid release. Weekly for 30 wk (beginning June 23, 1993) parasitoids were released in five individual Blattidae-infested (*P. americana*, *P. australasiae*, and *P. fuliginosa*) treeholes by placement of laboratory-parasitized oothecae into a parasitoid release chamber (Fig. 4.1) mounted near (≤ 60 cm) the entrance of each treehole. Before laboratory-parasitization, 1 to 14-d-old oothecae were killed by a 24 h freeze (-16°C) (for each release wk 1 through 12) or a dose of irradiation (2.5 kR) (for each release wk 13 through 30). After killing, oothecae were immediately (< 2 h) placed individually into 18-ml snap cap vials containing a single, 2- or 3-d-old female parasitoid, and incubated at 30°C for 28 d. After 28 d, oothecae were randomly divided into five groups, and each group randomly assigned to a treehole.

The release of parasitoids was accomplished by placing the laboratory-parasitized oothecae, hereafter termed "seeds", in the male side of a plumbers pipe adapter (Fig. 4.1E; Carlon Co., E943 H; 5.5 cm high (2 cm threaded), 5 cm i.d.); to the bottom of the male side was attached a screen (3 by 3 mm mesh) to secure the seeds, and to allow parasitoids to escape after emergence. The female half (Fig. 4.1D; 5.5 cm high (2 cm threaded inside), 5 cm i.d.), also screened, was fastened to the outside of a larger section of pipe (Fig. 4.1C; 6 cm o.d., 11 cm from apex of dome to bottom) open on the bottom but with a closed, dome-shaped apex onto which lay a piece of plywood (Fig. 4.1B; 1 cm thick, 21.5 by 21.5 cm) which had a hole (7 mm diam.) drilled through its diagonal center. A nylon cord (Fig. 4.1A) was guided through the plywood and a similar hole drilled in the apex of the domed-top of the large pipe (Fig. 4.1C); a triple-knot was then tied inside the dome to allow the entire apparatus to hang from the cord. A 30 cm spike was driven ca. 4-5 cm into the tree, and to its end was tied the loose end of the cord so that the entire unit was suspended above the ground and away from the tree.

After remaining in the field for 3 or 6 wk, seeds were returned to the lab, and the number from which parasitoids had emerged was counted. Parasitoid emergence was determined by the presence of an exit hole (0.5-1 mm diam.), and confirmed by opening all seeds. Presence of a parasitoid emergence hole was not always indicative of total parasitoid emergence; sometimes only a few parasitoids would emerge, leaving most inside, where they would eventually die. When this occurred, the seed was counted as having not produced parasitoids. Also, sometimes a parasitoid emergence hole was not evident, but by opening each seed it could be determined which ones produced a clutch of

parasitoids; the inside of a seed from which parasitoids had emerged was clean, and devoid of embryonic material. By opening each seed and observing from the inside out, the parasitoid exit hole often became evident.

Estimates of the number of female parasitoids released per treehole for the 30 wk test were based on a mean of 53.2 female A. hagenowii per clutch (Table 4.5) from irradiated 3 to 11-d-old oothecae. This value was used to calculate the maximum number of female parasitoids which could have emerged, per treehole microhabitat, had all oothecae producing parasitoids produced a female-biased clutch. This, however, is often not the case; a percentage of oothecae will produce an all-male clutch. Therefore, the number of female parasitoids which could have been available had only 80, 60, and 40% of oothecae producing parasitoids produced a female-biased clutch (i.e. 20, 40, and 60% of the oothecae produced an all-male clutch) was also calculated.

Parasitism of sentinel oothecae. To monitor the activity and biological control potential of A. hagenowii at natural and inflated (i.e. inundative release) populations, the parasitism of sentinel P. americana oothecae was followed weekly for one year near (≤ 30 cm) the entrance of 25 Blattidae-infested treeholes on the University of Florida campus beginning on April 13, 1993. Table 4.1 provides, for each treehole, the wk sentinel oothecae were used, the number of sentinel oothecae used, and the percent total recovery of sentinel oothecae. Parasitism was monitored by placing 3, <14-d-old oothecae into an ootheca-holding device (OHD; Fig. 4.2). Oothecae were replaced after 1 wk. Exposed oothecae were returned to the lab, and held in 18-ml snap cap vials at 28-30°C for (1) 45 d, or (2) until A. hagenowii or cockroach nymphs emerged, whichever was first; the fate

of each ootheca was recorded as having produced parasitoids, cockroaches, or neither. The oothecae from one OHD were held together in the same vial. There were 1-3 OHDs placed per treehole.

Each OHD was stabilized by a 171 gm pyramid-shaped, lead fishing weight (Fig. 4.2K); attached to the weight was ca. 30 cm of 60 lb. test monofilament fishing line (Fig. 4.2B). The monofilament line was guided through the inverted plastic cap from a 20 ml liquid scintillation vial (Fig. 4.2J; 1.3 cm high, 2.7 cm diam.; Wheaton Scientific, Millville, NJ) which had a 3 mm diameter hole drilled through its center top. Next was guided a disc of thin, pliable plastic (Fig. 4.2H; 5.5 cm diam.) cut from the top of a 237 ml cup (Sweetheart Plastics, Wilmington, MA). The disc served as a landing and crawling platform for parasitoids. Onto the disc was placed, inverted, the round top (4.8 cm diam., 0.6 cm high) from a plastic souffle cup (Fig. 4.2G; 29.6 ml, Solo Cup Company, Urbana, IL) which served as the holder of the sentinel oothecae (Fig. 4.2I). Another scintillation vial cap (Fig. 4.2F) was then placed, dorsum up, into the souffle cup top to serve as a spacer for the roof of the OHD. On top of the cap was placed the dome-shaped bottom (i.e. roof) (Fig. 4.2E; 6.5 cm diam.) from a 237 ml plastic cup (Sweetheart Plastics, Wilmington, MA). The hole in the apex of the dome was sealed, to prevent water seepage into the ootheca-holding well, with a 2 by 2 cm patch of rubber from a bicycle tube (Fig. 4.2D) secured by the weight of a 43 gm barrel-shaped, lead fishing weight (Fig. 4.2C). The loose end of the monofilament line was then secured to the end of a steel spike (Fig. 4.2A; 13 cm long) driven ca. 3 cm into the tree. The OHD was constructed so that all the components of the mechanism, parts C through J inclusive, could slide on the monofilament line.

Results

In the RCBD experiment, a significantly greater percentage of irradiated-killed and control oothecae produced a female-biased clutch than oothecae killed by freezing; this was the result of *A. hagenowii*'s inability to develop in some frozen-killed oothecae (Fig. 4.3). The proportion of oothecae from which a female-biased clutch emerged for irradiated-killed and control treatments was not significantly different.

Analysis of variance among treatments in the RCBD experiment indicated no difference in the number of female ($P=.4016$) and male ($P=.8181$) progeny per clutch, arcsine of the square root of the sex ratio ($P=.7460$), and developmental time ($P=.5569$) (Tables 4.2 and 4.3). Therefore, by pooling all data ($n=35$ clutches), the mean (95% confidence interval) number of female and male progeny per clutch, sex ratio, and developmental time was 68.5 females (64.7-72.3), 7.1 males (5.7-8.5), 0.091 male (.080-.103), and 28.7 d (28.2-29.2).

For each factorial analysis of variance, only age was significant (Table 4.4). Therefore, the effect of age on the number of emergent females and the log of the number of emergent males per clutch was analyzed, over all doses, by pairwise comparison of adjusted treatment means (LSMEANS age / Pdiff; in SAS) for clutches (Table 4.5). Significantly more female parasitoids emerged from 3 to 11- and 12 to 18-d-old oothecae than from 19 to 25-d-old oothecae. Although the number of emergent females from 3 to 11- and 12 to 18-d-old oothecae was not significantly different at $\alpha=.05$, the P value (.0747) was small enough to indicate the likelihood of a significant difference. The number of emergent males from 3 to 11-d-old oothecae was significantly greater than the number from 12 to 18- and 19 to 25-d-old oothecae.

During the 30 wk release program there was a total of 1,399 seeds placed in each of the 5 release treeholes, with the number ranging from 10 to 111 per treehole per wk (median=47, mode=30), depending on availability. For the first 12 wk, seeds were killed by freezing (-16°C) before being laboratory-stung, incubated, and distributed among the five release treeholes; $19.6 \pm 3.0\%$ (mean $\pm$ SE) ($n=60$; 12 wk $\times$ 5 treeholes) of the frozen-killed seeds placed per wk per treehole produced a clutch of parasitoids. For the remaining 18 wk seeds were killed by irradiation (2.5 kR); $26.2 \pm 3.7\%$ ($n=90$; 18 wk $\times$ 5 treeholes) of the irradiated-killed seeds placed per wk per treehole produced a clutch of parasitoids.

Over the entire 30 wk release period, in treeholes 1, 2, 6, 7, and 10 there were 379, 353, 373, 382, and 356 seeds, respectively, from which parasitoids emerged, or 27.1, 25.2, 26.7, 27.3, and 25.5% of the total number of seeds (1,399) placed per treehole. These percentages resulted in comparable per treehole estimates of the number of female parasitoids released (Table 4.6). Assuming all emergent clutches were female-biased with a mean of 53.2 females per clutch, the maximum number of female parasitoids released (20,322) was in treehole 7, while the lowest (18,780) was in treehole 2.

In treeholes where parasitoids were released, sentinel ootheca parasitism for the 10 wk period July 28 through September 29, inclusive, (6th-15th wk of release) was 70-100%, and 50-80% on July 7, October 20, November 10, and December 1, representing the 3rd, 18th, 21st, and 24th wk of release, respectively (Fig. 4.4). Sentinel ootheca parasitism in control treeholes reached its peak on July 7, when it was 46%. Control parasitism was between ca. 10 and 40% during 26 of the 32 wk from April 6 through November 10; parasitism on the remaining 6 wk was ca. 5-10%. As expected, parasitism

decreased during the fall and into the winter months. Parasitism rose above 10% only twice (12% and 17%) during the 20 wk period from November 17 through March 30 (1994), and was 0% on six of those wk.

Parasitism of sentinel oothecae in treeholes where parasitoids were released was higher than in control treeholes each wk after the parasitoid release program was initiated on June 23. Furthermore, parasitism of sentinel oothecae in release treeholes was higher than in the control treeholes for 10 of the 11 wk after the parasitoid release program was terminated.

Discussion

A principal requirement for the inundative field-release of parasitoids from laboratory-stung hosts is to prevent further infestation from unsuccessful laboratory-parasitism. Morgan et al. (1986) prevented Musca domestica L. adults from emerging by irradiating 2-d-old pupae with a 20 kR dose of gamma irradiation. Furthermore, exposing 2-d-old pupae to Spalangia endius within 2 h after they had been irradiated with 25 kR resulted in 95% successful parasitism. Irradiated pupae could also be stored at refrigerator temperatures for several wk and remain acceptable to female parasitoids. Parasitism of irradiated pupae stored at 15°C in paper cartons was ca. 80-90% for each of the first 5 wk of storage, but was <36% for each of the next 5 wk. Storage of irradiated pupae (15°C) in small heat-sealed glass containers in a plain air or nitrogen atmosphere provided 2-3 more wk of shelf-life, as successful parasitism remained above 70% through the seventh (nitrogen atmosphere) and eighth (air atmosphere) wk of storage. Parasitism of pupae held at room temperature rapidly decreased to 2% after just 2 wk.

In the present study, ≥ 2.5 kR irradiation or 24 h at -16°C killed 100% of exposed *P. americana* oothecae. Per ootheca production of female *A. hagenowii* from irradiated- and frozen-killed oothecae was not significantly different (Table 4.3), but the proportion of irradiated-killed oothecae that yielded a female-biased clutch was twice that of frozen-killed oothecae (Fig. 4.3). From a practical standpoint for field-release, the advantage of parasitoid development in killed oothecae is obvious. Release of parasitoids in the field from laboratory-parasitized oothecae can be achieved without the threat of accidental infestation from unsuccessful laboratory parasitization of viable oothecae. Furthermore, radiation dose did not affect the number of female parasitoids produced per ootheca ($P=.7761$) in the factorial experiment, but the age of the ootheca did ($P=.0012$) (Table 4.4). In Chapter III, the randomized block design showed a borderline difference in the number of females per clutch ($P=.0997$) (Table 3.4) among 3 ages of oothecae, again suggesting a possible age effect on the number of females per clutch. Differential female production could be the result of several factors. First, it is known (Heitmans et al. 1992) that the probability of offspring survival increases with clutch size in *A. hagenowii*. If mothers deposit a greater number of eggs per oviposition in younger oothecae, this might account for the larger number of emergent females in younger hosts. One way to document this would be to dissect and count the number of eggs remaining in the ovaries of females that had oviposited in different aged oothecae. The combination of egg counts with emergent progeny might elucidate the difference. Also, there may be an age-related physical or physiological constraint of the host on the parasitoid affecting the number of emergent females.

Parasitism of sentinel oothecae in release treeholes was higher than in the control treeholes for 10 of the 11 wk after the parasitoid release program was terminated. The final date of parasitoid release was January 19, 1994 and, as stated earlier, seeds remained in the field for 6 wk to allow parasitoids to emerge, as development of A. hagenowii is slowed by cool temperatures (Narasimham 1984); ca. 26% of the 22 seeds, or about 5 seeds per treehole, produced parasitoids during the final 6 wk period. In laboratory studies, females of the Gainesville, Florida strain of A. hagenowii survived 3.4 and 7.3 d when unfed and honey-fed, respectively (Hagenbuch et al. 1988). Conservatively then, if all parasitoids placed in the field on January 19 emerged during the last wk (February 23 to March 2), all would have died by March 9. Parasitism of sentinel oothecae from March 9 forward then, would be the result of naturally occurring parasitoid populations. In treeholes where parasitoids were released (Fig. 4.4), parasitism of sentinel oothecae remained higher than in control treeholes for the 4 wk period from March 9 through March 30. This is a good indication that parasitoids from naturally occurring Blattid oothecae, parasitized during the release program, served as inoculum for sentinel oothecae during that 4 wk period. Unfortunately, sentinel ootheca parasitism monitoring was terminated on April 6.

The biological control of urban pests has recently received a great deal of attention. Increased legislation resulting in the banning of certain active ingredients, the high cost of organic pesticide development, pest resistance, and the lack of active ingredients with new modes of action are all driving the structural pest control industry to adopt less toxic, exposure minimizing control technologies. Here, and elsewhere

(Hagenbuch et al. 1989, Pawson & Gold 1993), the potential of A. hagenowii as a biological control agent has been documented. A method has been developed whereby oothecae can be killed, laboratory-stung, and distributed in the field for mass-release of A. hagenowii in biological control programs of Blattid cockroach species.

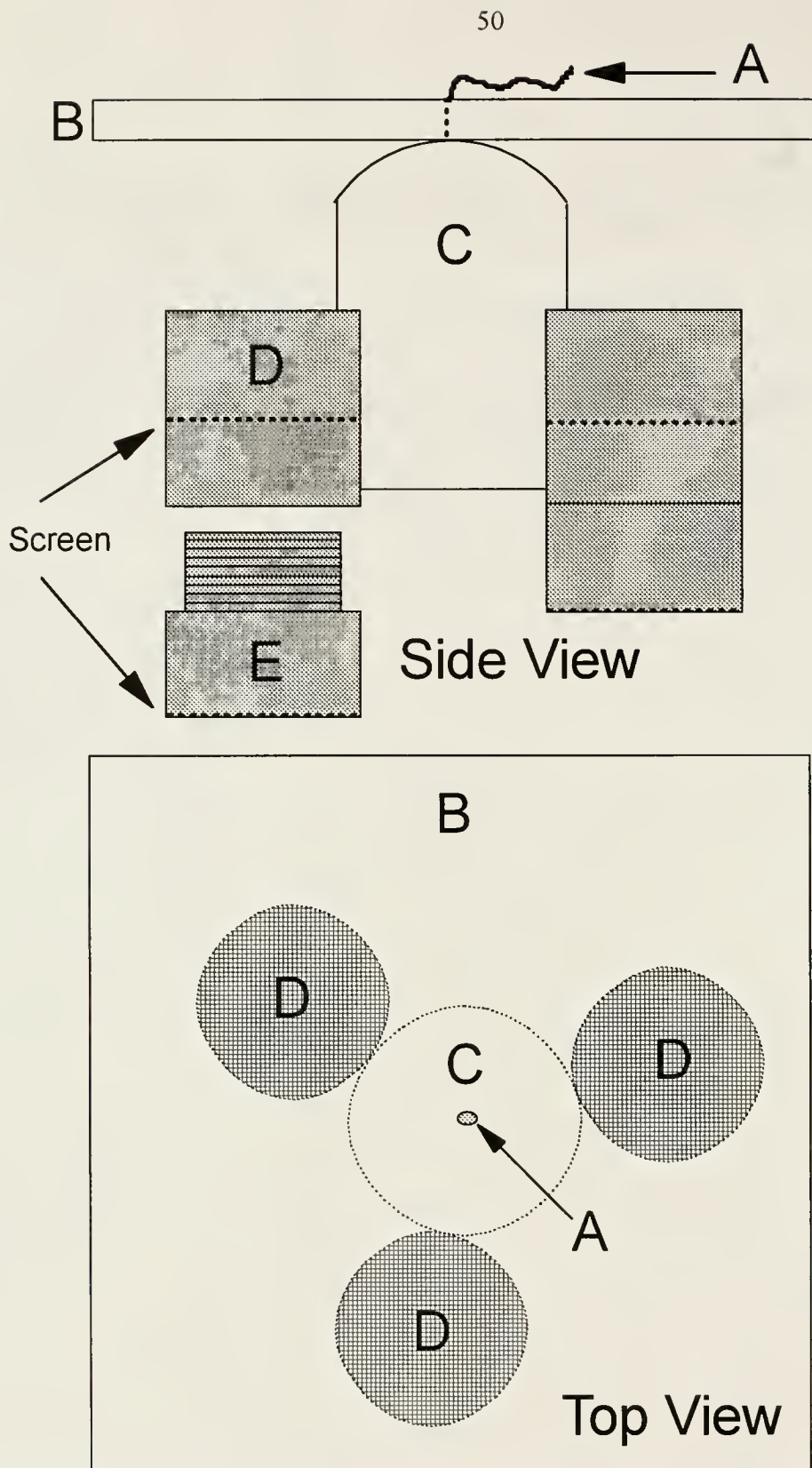


Fig. 4.1. Parasitoid release chamber for inundative release of *A. hagenowii* in treehole microhabitats. A, cord; B, plywood roof; C, large pipe; D, female half; E, male half. Scale is one-half.

Table 4.1. For 25 treeholes, the weeks sentinel ootheca parasitism was monitored, the total number of sentinel oothecae used, and the overall percent recovery of sentinel oothecae from each treehole.

Treehole	Weeks sentinel oothecae were used	# Sentinel oothecae used	Recovery (%) of sentinel oothecae ^b
1 ^a	1-52	120	93.3
2 ^a	1-52	282	84.4
3	1-52	276	81.2
4	1-52	270	80
5	1-52	117	90.6
6 ^a	1-52	138	95.7
7 ^a	1-52	126	86.5
8	1-52	291	93.8
9	1-52	129	95.3
10 ^a	1-52	135	95.6
11	1-33	72	91.7
12	1-52	285	86.3
13	1-52	102	94.1
14	1-9	54	96.3
15	1-52	120	94.2
16	1-52	135	99.2
17	1-52	138	97.8
18	1-52	273	89.4
19	7-45	72	79.2
20	7-52	87	89.7
21	7-52	96	78.1
22	7-52	111	91
23	11-21	9	66.7
24	11-52	63	92.1
25	12-52	204	81.9

^a Parasitoids were released weekly for 30 wk, from June 23, 1993 to January 19, 1994.

^b % Recovery of sentinel oothecae for a treehole = total # sentinel oothecae returned to the laboratory divided by the total # sentinel oothecae used in that treehole (i.e. 3rd column of table).

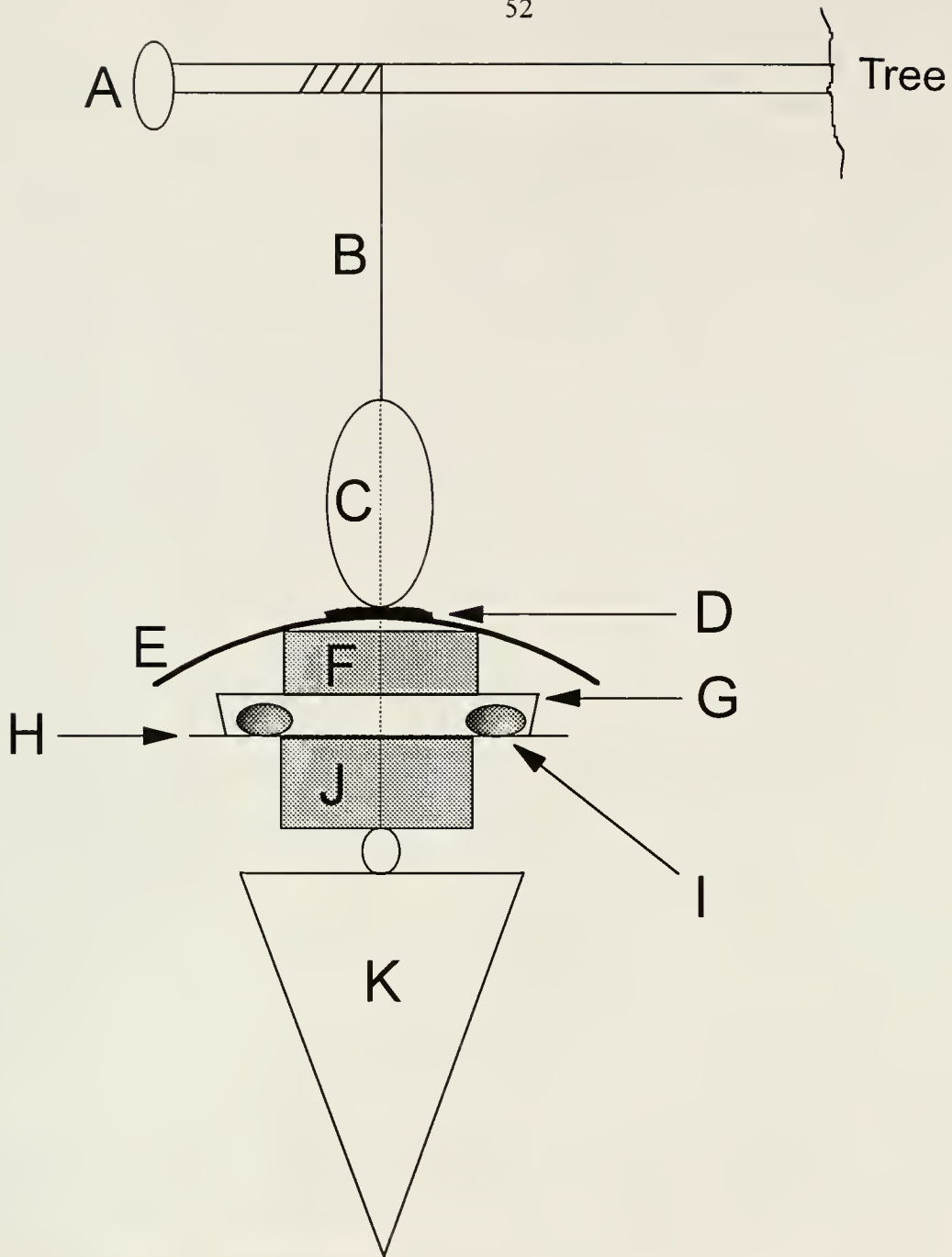


Fig. 4.2. Ootheca-holding device for use in monitoring sentinel ootheca parasitism in treehole microhabitats. A, 13 cm spike; B, 60 lb. test monofilament; C, 43 gm barrel weight; D, rubber tubing; E, roof; F, spacer cap; G, inverted soufflé cup top; H, plastic disc; I, ootheca; J, inverted cap; K, 171 gm pyramid weight. All components of the device are actual size.

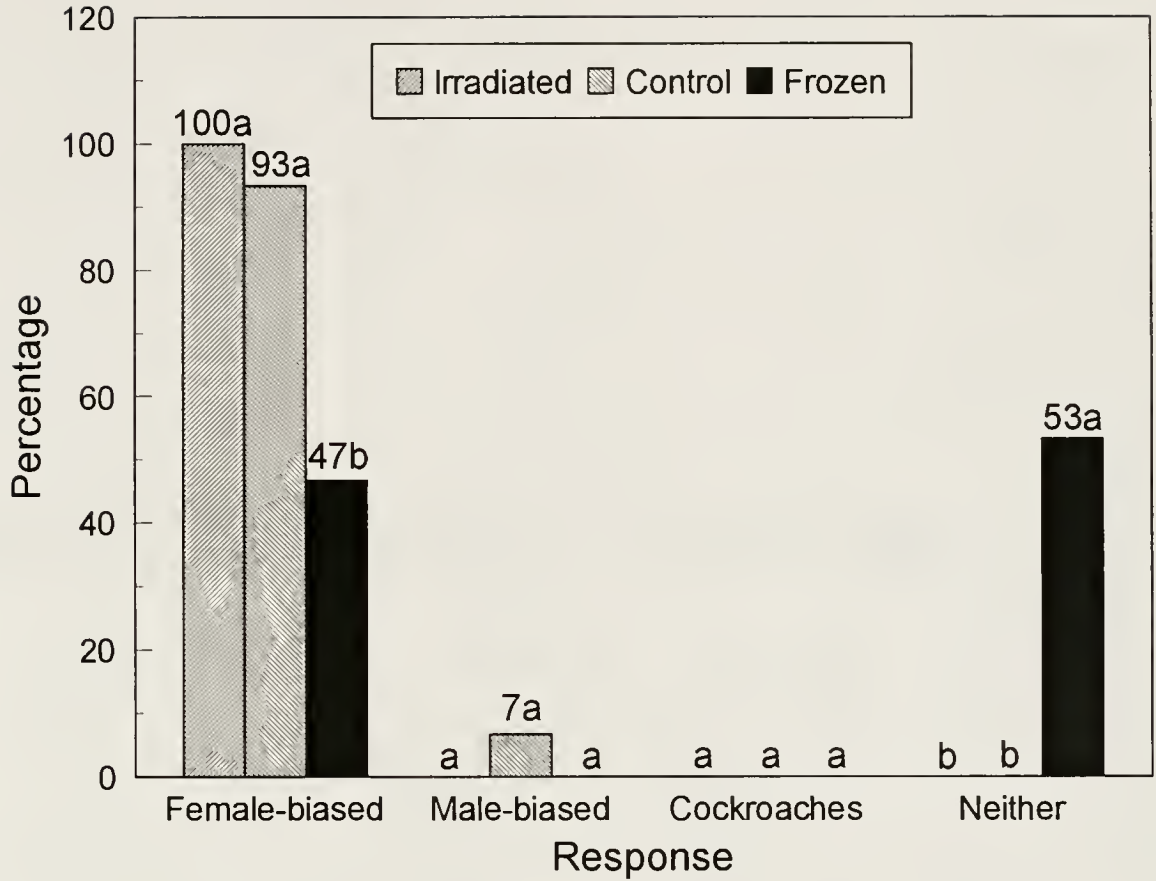


Fig. 4.3. Percentage of oothecae from which emerged (1) a female-biased clutch of parasitoids, or (2) a male-biased clutch of parasitoids, or (3) cockroaches, or (4) neither parasitoids nor cockroaches in the RCBD experiment. Chi-square Homogeneity of Proportions test (Ott 1988, p.253): $X^2=21.2$, $df=6$, $P<.005$. For each response, bars separated by different letters are significantly different. Proportions within each response were separated by non-inclusion of zero in a 95% confidence interval constructed on the difference in proportions between treatments (i.e. pairwise comparisons of treatment proportions within each response) (Hochberg & Tamhane, p. 275 for formula).

Table 4.2. Analysis of variance for the number of female and male progeny per clutch, arcsine of the square root of sex ratio, and developmental time (RCBD experiment).

Dependent Variable	Source ^a	Sum of Squares	Mean Square	F Value	P Value
# Females	Treatment	180.378	90.189	0.94	0.4016
	Clutch	1684.570	842.285	8.79	0.0010
	Error	2876.195	95.873		
	Total	4741.143			
# Males	Treatment	5.955	2.977	0.20	0.8181
	Clutch	183.981	91.990	6.24	0.0054
	Error	441.950	14.732		
	Total	631.886			
Arcsine	Treatment	0.00183	0.00092	0.30	0.7460
	Clutch	0.03032	0.01516	4.90	0.0144
	Error	0.09282	0.00309		
	Total	.12497			
Time	Treatment	3.150	1.575	0.60	0.5569
	Clutch	10.845	5.423	2.06	0.1457
	Error	79.147	2.638		
	Total	93.143			

^a For each analysis of variance, the degrees of freedom for Treatment, Clutch, Error, and Total are 2, 2, 30, and 34, respectively.

Table 4.3. Number of female and male *A. hagenowii* per clutch, sex ratio, and developmental time from irradiated-killed, frozen-killed, and unkilld *P. americana* oothecae (RCBD experiment).

Treatment	n	Response Variable ^{a,b}			
		Number females/clutch (mean±SE)	Number males/clutch (mean±SE)	Sex ratio/clutch (mean & 95% CI)	Development time/clutch (d) (mean±SE)
Irradiation	15	66.4 a ± 3.0	7.5 a ± 1.5	.095 a (.072-.121)	28.7 a ± 0.5
Freezing	7	66.9 a ± 4.5	6.7 a ± 1.0	.091 a (.071-.113)	29.3 a ± 0.4
Control	13	71.5 a ± 3.2	6.9 a ± 0.8	.087 a (.080-.095)	28.5 a ± 0.3

^a Means within a column followed by the same letter are not significantly different.

^b See Table 4.2 for analysis of variance of each variable.

Table 4.4. Analysis of variance for the number of female progeny per clutch, and the log of the number of males per clutch (3 x 4 factorial experiment).

Dependent Variable	Source ^a	Sum of Squares	Mean Square	F Value	P Value
# Females	Dose	223.937	74.646	0.37	0.7761
	Age	2915.469	1457.734	7.19	0.0012
	Dose*Age	641.144	106.857	0.53	0.7867
	Clutch	15735.926	2247.989	11.09	0.0001
	Error	22098.241	202.736		
	Total	41900.305			
Log (# males)	Dose	0.34585	0.11528	1.88	0.1371
	Age	0.52112	0.26056	4.25	0.0167
	Dose*Age	0.39835	0.06639	1.08	0.3770
	Clutch	1.33946	0.19135	3.12	0.0048
	Error	6.68069	0.06129		
	Total	9.64060			

^a For each analysis of variance, the degrees of freedom for Dose, Age, Dose*Age, Clutch, Error, and Total are 3, 2, 6, 7, 109, and 127.

Table 4.5. Means separation by pairwise comparison of adjusted treatment (i.e. age) means (LSMEANS in SAS) for clutches for the number of females per clutch and the log of the number of males per clutch.

Age (d)	n	Number (mean) per clutch	
		Females ^a	Males ^b
3-11	48	53.2 a	5.3 a
12-18	44	47.9 a	3.9 b
19-25	36	41.2 b	3.8 b

^a Pairwise comparison of LSMEANS: 3-11 vs 12-18, $P=.0747$; 3-11 vs 19-25, $P=.0002$; 12-18 vs 19-25, $P=.0386$.

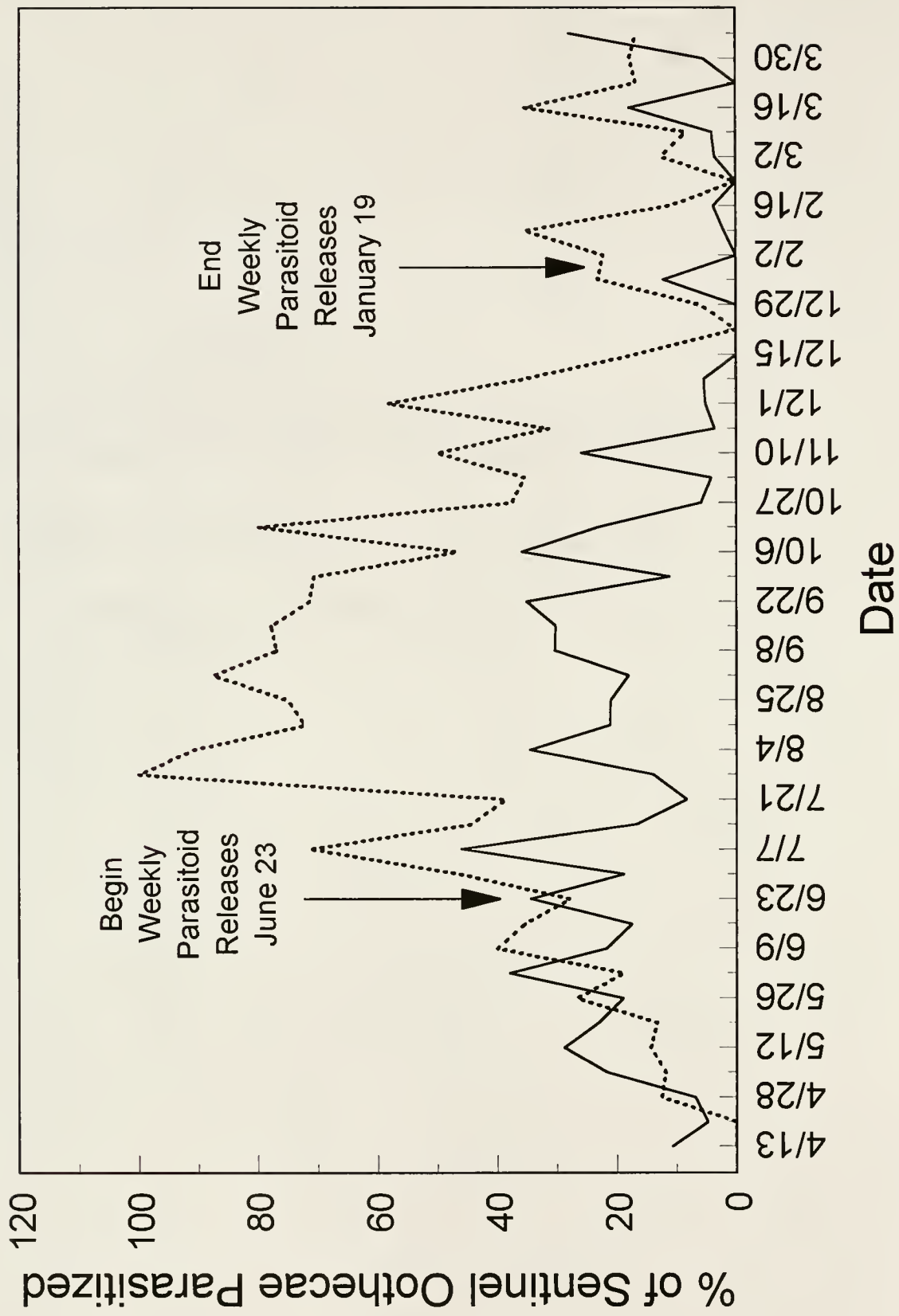
^b Pairwise comparison of LSMEANS: 3-11 vs 12-18, $P=.0113$; 3-11 vs 19-25, $P=.0085$; 12-18 vs 19-25, $P=.8277$.

Table 4.6. Estimated numbers of *A. hagenowii* females released in 5 treeholes over 30 wk.

% Female-biased clutches ^a	# Females released per given treehole				
	1	2	6	7	10
100	20,163	18,780	19,844	20,322	18,939
80	16,130	15,024	15,875	16,258	15,151
60	12,098	11,268	11,906	12,193	11,364
40	8,065	7,512	7,937	8,129	7,576

^a Percentage of the total number of parasitoid-producing oothecae from which a female-biased clutch emerged. Female production estimates are based on a mean of 53.2 female parasitoids per clutch (Table 4.5). For example, for treehole 1 there were 379 oothecae from which parasitoids emerged. If 100% of clutches were female-biased, then ca. 20,163 (379×53.2) female parasitoids were released during the 30 wk period. If only 80% of the 379 oothecae produced a female-biased clutch, then 16,130 females were released, assuming the remaining 20% of clutches were all-male.

Fig. 4.4. Parasitism of sentinel *P. americana* oothecae in treeholes where parasitoids were released weekly for 30 wk (dashed line) during a 31 wk period (June 23, 1994 to January 19, 1994), and control treeholes where parasitoids were not released (solid line). Sentinel oothecae placed in the field on August 4, October 6, and November 17 remained in the field for 2 wk, while those placed in the field on January 5 remained in the field for 4 wk.



CHAPTER V
A KAIROMONE FOR Aprostocetus hagenowii (Ratzeburg), A
EULOPHID PARASITOID OF
AMERICAN COCKROACH (Periplaneta americana (L.)) OOTHECAE

Introduction

Host-location, or finding, by insect parasitoids has been reviewed by several authors (Vinson 1976, Weseloh 1981). Weseloh (1981) defined chemical host-location as the discovery, or perception, of a host from a distance due to short- and long-range volatile compounds (i.e. kairomones) produced by the host, its activity, or one of its products. Host-location by short-range kairomones has been well documented, providing most of the host-location literature. Commonly, short-range kairomones are found on or emanating from the host or one of its products. Eggs (Leonard et al. 1987), pupae (Carde & Lee 1989), larvae (Vinson et al. 1975), wing scales (Jones et al. 1973, Shu et al. 1990), and frass (Jones et al. 1971, Nemoto et al. 1987a,b, Auger et al. 1989, Fukushima et al. 1989) contain compounds which elicited host-finding and host-acceptance behaviors from parasitoids. Vinson et al. (1975) identified several methyl-branched, saturated C₃₂, C₃₃, and C₃₄ hydrocarbons from larvae of Heliothis virescens which elicited host-searching behaviors from Cardiochiles nigriceps. Jones et al. (1973) and Shu et al. (1990) showed that tricosane and methyl-branched hydrocarbons found in moth scales attract the egg parasitoids Trichogramma evanescens and T. nubilale, respectively. Frass also contains chemical attractants for parasitoids; both volatile and contact host-location kairomones

have been identified from host frass. Jones et al. (1971) identified 13-methylhentriacontane in the frass of H. zea; this hydrocarbon triggered short-range, host-seeking behaviors by the parasitoid Microplitis croceipes. Parasitoids characteristically antennate host frass and alter their search behavior when they contact it.

After locating a host, a parasitoid will examine it, and accept or reject it based on chemical and physical criteria. Host examination leading to acceptance or rejection commonly involves antennal drumming behaviors mediated by tactile compounds. For example, Aprostocetus hagenowii (Ratzeburg), a Eulophid parasitoid of oothecae of cockroaches in the family Blattidae, exhibited typical host-recognition behaviors (drumming, tapping, drilling) on glass beads treated with calcium oxalate from colleterial glands and mucopolysaccharides from salivary glands of Periplaneta americana (L.), the American cockroach (Vinson & Piper 1986). Comperia merceti (Compere) uses the ootheca-fixing cement from the cockroach Supella longipalpa as a host-acceptance kairomone (Van Driesche & Hulbert 1984).

The biology of A. hagenowii has been well documented (Roth & Willis 1954, 1960, Cameron 1955, Edmunds 1955, Narasimham 1984). Its preferred host is oothecae of P. americana, but it will also parasitize the oothecae of P. australasiae (F.) (Australian), P. fuliginosa (Serville) (smokybrown), P. japonica Karny (Japanese), P. brunnea Burmeister (brown), Blatta orientalis L. (Oriental), Eurycotis floridana (Walker) (Florida woods), and E. biolleyi Rehn.

Upon emergence from the ootheca, parasitoids sibmate and can immediately oviposit. If parasitized by a single foundress, an ootheca will produce ca. 51 female and 7

male progeny per clutch after a 31-35 d developmental period at 30°C (Chapter III). The sex ratio of this parasitoid is strongly female-biased (86%). The broad host range, reproductive strategy, and life cycle of A. hagenowii make it an excellent candidate for the biological control of Blattidae. Until now, little was known concerning chemically-mediated host-location by A. hagenowii. In the present study, I investigated a host-location kairomone, for A. hagenowii, from P. americana oothecae and frass.

Materials and Methods

Parasitoid. A. hagenowii used in this study were 1-d-old (29 ± 11 h), unfed females that had emerged from 3-5 P. americana oothecae. The day preceding bioassay, parasitoids were released together into a clean Plexiglass cage (Hagenbuch et al. 1988), and the empty oothecae removed to avoid possible habituation to the kairomone. The inside of cages were wiped once every 6 wk with ca. 10% isopropyl alcohol, and air dried before reuse. See Chapter III for details concerning A. hagenowii and P. americana colonization and colony maintenance. In short, daily 24, 14-d-old oothecae from field-collected P. americana were placed individually into 18-ml plastic vials containing a single 2- or 3-d-old female parasitoid and incubated at 28-30°C.

Olfactometer. The Y-tube olfactometer described by Vander Meer et al. (1988, includes sketch) for the isolation of Solenopsis invicta Buren trail pheromone was used in this study. In short, the barbed ends of a glass, Y-shaped hose connector (0.6 cm i.d; Kontes, Vineland, NJ) were replaced with glass tubing. The resulting common arm was 7.5 cm. Each lateral arm extended 5 cm before penetrating ca. 1.5 cm into the male end of a 24/40 ground glass joint. Another section of glass tubing penetrated ca. 1.5 cm into and

4 cm out of the female half of the joint, resulting in a 16 cm insect trap comprised of the joint; total lateral arm length was 25 cm.

A chamber, constructed from the top half of a 50 ml Nalgene squirt bottle (3 cm i.d., 1 cm i.d. mouth), was built to allow the unprovoked, natural migration of parasitoids into the common arm during a bioassay. Into the bottom of the chamber was fitted stainless steel screen (150 mesh; Small Parts, Miami, FL) to prevent parasitoids from escaping and to allow the free flow of air from the common arm. Parasitoids were transferred from the Plexiglass holding cage into the chamber through its mouth, which was then fitted to the end of the common arm.

Air for all bioassays was breathable quality compressed air which was humidified (ca. 75% R.H.) over 100-200 ml of saturated NaCl (99.5%; Sigma, St. Louis, MO) before being regulated through two flowmeters (65 mm variable area, stainless steel float, 333 ml/minute maximum airflow; Cole Parmer, Niles, IL) mounted to the inside, back wall of a white box (1.25 cm thick plywood; 58 cm high by 74 cm wide by 69 cm deep) with an open front and top. The olfactometer was placed on the center floor and connected to the outlet tube from each flowmeter; a 3 cm space between the floor and the bottom of the back wall allowed the flowmeter outlet tubes to connect to the ends of the lateral arms of the olfactometer.

The air tube connecting the compressed air tank and the 0.5 L flask housing the saturated NaCl solution was corrugated Teflon FEP tubing (6.4 mm i.d., 250 max PSI; Cole Parmer, Niles, IL) with 2.54 cm non-corrugated ends. An identical tube connected the flask to a 90° Teflon tee connector (7 mm i.d.) from which tubes connected to each

flowmeter inlet; each inlet was equipped with a Teflon, straight male pipe adapter (6 mm o.d.). The outlet from each flowmeter was also equipped with a Teflon, straight male pipe adapter (10 mm o.d.); corrugated Teflon FEP tubing (10 mm i.d.) connected each flowmeter outlet with the corresponding lateral arm of the olfactometer.

Ootheca bioassay development. The bioassay was developed by placing a single, pristine (i.e. not dented, and without attached detritus), <10-d-old *P. americana* ootheca in the distad end of a lateral arm of the olfactometer and recording the number of parasitoids, of 10 placed in the chamber, that chose the ootheca or the sterile air (i.e. the lateral arm opposite the ootheca), and those that did not make a choice, after 10 minutes at each of four airflow rates (30, 60, 120, and 240 ml/minute/flowmeter); the procedure was repeated but with the ootheca in the other lateral arm of the olfactometer. Each run was conducted with a unique cohort of 10 parasitoids.

A successful "choice" was the event whereby a parasitoid migrated up a lateral arm and into the olfactometer trap created by the 24/40 ground glass joint (see sketch, Vander Meer et al. 1988); at this point parasitoids could not retreat. A bioassay replicate was the summation of parasitoids from both lateral arms for each response, at each airflow rate. There were 6, 9, 5, and 5 bioassay replicates, or 120, 180, 100 and 100 parasitoids bioassayed, for the 30, 60, 120, and 240 ml/minute/flowmeter airflow rates, respectively. After each run of 10 parasitoids, the olfactometer was disassembled, rinsed with acetone (99.9%; Baxter, Muskegon, MI), and fan-dried for 3 to 5 minutes.

The response of parasitoids to sterile air (i.e. no ootheca) from both lateral arms was investigated at the highest airflow rate. All bioassays were conducted between 0900 and 1700 h, and 28-31°C.

Frass bioassay. After the bioassay for oothecae had been developed, 50, 100, and 200 mg samples (n=2, 3, and 2 bioassay replicates, respectively) of 1 to 5-d-old frass from mixed sex adult and large nymphal P. americana (see Chapter III for cockroach origin, diet, and rearing) was also bioassayed for its attractiveness to A. hagenowii.

Isolation of kairomone from oothecae. On two occasions 30, <10-d-old P. americana oothecae were immersed in successive 10, 5, 2, and 2 minute hexane (99.9%; Baxter, Muskegon, MI) washes to remove cuticular lipids. On both occasions, the 4 washes were combined, reduced under a gentle stream of nitrogen to ca. 0.5 ml, and chromatographed on a silica gel (70-230 mesh; Sigma, St. Louis, MO) packed disposable Pasteur pipette column (5 cm long bed), wetted with hexane, by gravity elution with sequential 6-ml aliquots of 0, 1, 5, 10, and 30% anhydrous ether (99.9%; Aldrich, Madison, WI) in hexane (Carroll 1961). Each fraction was dried under nitrogen and immediately (<1 minute) reconstituted in hexane to provide one ootheca equivalent (OE) per 10 ul. For each fraction, an OE was pipetted onto a paper wick (3 by 14 mm; Northfork, Tumwater, WA) and bioassayed. For each OE tested, a paper wick control wetted with 10 ul hexane was placed in the opposite lateral arm of the olfactometer. As before, a bioassay replicate was the response of 20 parasitoids (i.e. 10 bioassayed for one OE in each lateral arm).

For the first separation, there were 4, 4, 3, 3, and 3 bioassay replicates for the 0, 1, 5, 10, and 30% ether in hexane fractions, respectively; for the second separation there were 3, 3, 3, 3, and 2 bioassay replicates. Each bioassay replicate was expressed as a percentage of the activity of one ootheca bioassay replicate on the day fractions were bioassayed (i.e. on the same day, the number of parasitoids responding to a fraction divided by the number responding to the ootheca). The activities were combined for each fraction, and reported as mean $\pm$ SE. When more parasitoids responded to a fraction than the ootheca, the response was treated as >100%.

After biological activity was discovered in the hydrocarbon-containing hexane fraction above, oothecal hydrocarbons were separated and bioassayed. Thrice, ootheca lipids were extracted and total hydrocarbons collected by column elution with a 6-ml aliquot of 1% ether in hexane (Brill & Bertsch 1985). The 1% fraction was concentrated and chromatographed on 10% AgNO₃-impregnated silica gel (200+ mesh, 3 cm long bed; Aldrich, Milwaukee, WI) by elution with 6-ml aliquots of 0, 2, and 10% ether in hexane to separate saturated and unsaturated hydrocarbons (Carlson & Service 1980). The resulting fractions were concentrated and chromatographed again through a 1 to 2 cm bed of silica gel by elution with 1 to 2 ml of hexane to remove potentially disruptive silver. Fractions were dried, immediately reconstituted in hexane to provide one OE per 10 μ l, and bioassayed as before.

For 2 of the separations, there was 1 bioassay replicate for each of the 0, 2, and 10% ether in hexane fractions; for the third separation, there were 4, 4, and 7 bioassay replicates for each of the 3 fractions.

Hydrocarbon analysis from oothecae and frass. Total hydrocarbon from oothecae and frass were analyzed by gas chromatography (GC), as were ootheca hydrocarbon fractions from AgNO₃-impregnated silica gel. Frass hydrocarbons were obtained by immersion of 200 to 600 mg in hexane for 24 h. Frass pellets were then ground with a glass rod, rinsed with 3-5 additional aliquots of hexane, concentrated, and chromatographed on silica gel by elution with 1% ether in hexane (Brill & Bertsch 1985). Samples were dried and immediately reconstituted in hexane to provide one mg equivalent of frass per μ l. Additionally, the quantity of 6,9-heptacosadiene was determined for oothecae and frass (n=4 independent separations for each, 1 quantification per separation) by coinjection of 0.1 μ g hexacosane standard and a known equivalent of total hydrocarbon from oothecae or frass.

Bioassay of adult female-derived 6,9-heptacosadiene. Fifty adult female *P. americana* of unknown age were placed in a jar (4 L) and washed with successive 100 ml aliquots of hexane for 10 and 15 minutes. The two washes were combined, rotoevaporated to ca. 5 ml, and chromatographed on silica gel (1 cm i.d. by 25 cm long bed) by elution with 80 ml 1% ether in hexane; ca. 5 female-equivalents were saved for total hydrocarbon analysis. Hydrocarbons from the remaining ca. 45 female-equivalents were separated on 10% AgNO₃-impregnated silica gel (1 cm i.d. by 15 cm long bed) by elution with 60-ml aliquots of 0, 2, and 10% ether in hexane. Silver was removed as before, fractions dried by rotoevaporation, reconstituted in 7 mls hexane, and stored at minus 16°C.

To further purify 6,9-heptacosadiene for bioassay, 5 female equivalents from the 10% ether fraction above were chromatographed again on a Pasteur pipette packed with 10% AgNO₃-impregnated silica gel as before, and analyzed with GC to obtain purity. The amount of alkadiene was then quantified, formulated in hexane to provide ca. one OE per 10 ul, and bioassayed (n=4 replicate bioassays).

Volatile collection from oothecae. Volatile hydrocarbons were collected on 20-30 mg Super Q adsorbent (Alltech, State College, PA) packed into the constriction of a disposable Pasteur pipette (VOLTRAP); the adsorbent was held in place on either end by glass wool. After packing, the adsorbent was cleaned by elution with 4-5 mls of hexane, and blown to dryness with a stream of nitrogen. The ootheca-holding chamber (OHC) was a glass tube (1.22 m by 0.6 cm i.d.) into which 175 pristine, 1 to 7-d-old oothecae were placed. At the airflow outlet of the OHC was attached a sleeve of heat-shrinkable Teflon tubing that extended ca. 2 cm past the end of the OHC. The airflow inlet of the VOLTRAP was fitted into the 2 cm extension so that it became part of the OHC. Charcoal gravel (#202292, GC hydrocarbon trap grade; Chromtec, Lake Worth, FL) was centrally packed between two, 2 cm long plugs of highly activated charcoal (Lewcott, Millbury, MA) in a glass tube (22 cm by 0.6 cm i.d.; CHARTRAP) to provide 13 cm of charcoal filter. The airflow inlet of the CHARTRAP was fitted into the Teflon FEP airflow outlet tube from an airflow meter (see "Olfactometer" section). To the airflow outlet of the CHARTRAP was attached a sleeve of Teflon tubing that extended 2 cm past the end of the CHARTRAP. The airflow inlet of the OHC was fitted into the 2 cm Teflon sleeve extension. Volatiles were collected from oothecae for 72 h using compressed air

(breathable quality) at an airflow rate of 7.212 liters/h, and 28-30°C. The VOLTRAP was eluted with ca. 0.5 ml hexane, concentrated to 100 ul, and analyzed by GC.

Gas chromatography. Because of the simplistic hydrocarbon composition of P. americana, hydrocarbons in this study were identified by chromatogram comparison with published identification of P. americana cuticular and hemolymph-derived hydrocarbons (Baker et al. 1963, Beatty & Gilby 1969, Jackson 1972), and retention time comparisons with n-alkane standards.

Hydrocarbon samples (in 1 or 2 ul hexane) were analyzed with a Tracor model 540 gas chromatograph equipped with a cool on-column injector, and a flame ionization detector held at 340°C. The capillary column was non-polar fused silica (DB-1; 30 m by 0.32 mm i.d., 0.25 micron thickness; J & W Scientific, Folsom, CA). Hydrogen was the carrier gas at a linear flow rate of 25 cm/sec. The temperature program began with a 2 minute hold at 60°C, then increased by 20°C per minute to 240°C with no hold time, then increased at 5°C per minute to 320°C and was held for 30 minutes. Data were recorded with Turbochrom 3 (PE Nelson, Cupertino, CA) analytical software with a PE Nelson 900 Series Interface.

Results

A. hagenowii was attracted to volatiles emanating from P. americana oothecae and frass. After 10 minutes, a significantly greater number of parasitoids (7.67) had responded to the ootheca at the highest airflow rate than at any of the lower airflow rates (Fig. 5.1A). Furthermore, at this airflow rate parasitoids invariably chose the ootheca over sterile air; when a parasitoid migrated up the Y-tube and made a choice, it chose the ootheca 99%

(i.e. number choosing ootheca divided by number choosing ootheca plus number choosing air) of the time (Fig. 5.1B). To determine the response of A. hagenowii to air movement, parasitoids were subjected to sterile air at the highest airflow rate, since this rate provided the best response to an ootheca. Parasitoids did not respond to sterile air; of 100 parasitoids (n=5 bioassay replicates) tested, only 1 migrated up the Y-tube. P. americana frass was also attractive to A. hagenowii; 50, 100, and 200 mg of 1 to 5-d-old frass were 97, 124.4, and 96.4% as active as an ootheca (i.e. on the same day, the number responding to frass divided by the number responding to an ootheca).

Once a suitable bioassay had been developed, the response of A. hagenowii to oothecal cuticular lipids was investigated. Bioassay of one OE of the fractions from silica gel clearly demonstrated that the majority of biological activity was in the hexane fraction (i.e. hydrocarbon), moderate activity in the 1% ether fraction, and virtually no activity of fractions containing more ether (Fig. 5.2A). The hexane and 1% ether fractions were, on average, 76.7 and 19.9%, respectively, as active as an ootheca. Oothecal hydrocarbons were then collected and separated on 10% AgNO₃-impregnated silica gel by elution with 0, 2, and 10% ether in hexane, and each fraction bioassayed as before. All the biological activity was in the 10% ether fraction (Fig. 5.2B); this fraction was 62.6% as active as an ootheca. Of 120 parasitoids bioassayed for each of the 0 and 2% ether fractions, 0 and 1 parasitoids, respectively, responded positively. For the 10% ether fraction, however, 86 out of 180 parasitoids responded positively.

GC analysis of total hydrocarbon from oothecae and frass were qualitatively (Fig. 5.3) and quantitatively (Table 5.1) very similar. Five peaks constituted 96 and 87% of the

hydrocarbons from oothecae and frass, respectively. The predominant hydrocarbon from both sources was 6,9-heptacosadiene (peak 3 in Fig. 5.3; after Baker et al. 1963, Beatty & Gilby 1969, Jackson 1972); it comprised 60 and 46% of all hydrocarbon from oothecae and frass, respectively.

Separation of oothecal hydrocarbons was accomplished by elution with 0, 2, and 10% ether in hexane (Fig. 5.4). Collectively, Figures 5.2 and 5.4 provide strong evidence that the hydrocarbon 6,9-heptacosadiene is the compound responsible for the attraction of *A. hagenowii* to *P. americana* oothecae. This compound accounted for 100% (13.2 mV) of the peak area in Fig. 5.4D. Though the alkadiene was present in the 2% fraction (ca. 0.28 mV; Fig. 5.4C), a vast majority was present in the 10% ether fraction (97.9% of all the 6,9-heptacosadiene collected). The 0% fraction contained only saturated hydrocarbons.

On a per mg fresh weight basis, frass contained ca. 37.1 times the amount of 6,9-heptacosadiene as oothecae (cuticular only). An ootheca contained ca. 2.09 ± 0.30 ug 6,9-heptacosadiene, or 0.024 ± 0.003 ug per mg fresh weight (based on an oothecal weight of 87.9 ± 1.29 mg, $n=9$). Frass, on the other hand, contained 0.881 ± 0.10 ug of alkadiene per mg fresh weight.

To confirm the biological activity of 6,9-heptacosadiene, the collection and separation of hydrocarbon from adult female cockroaches was made for bioassay. GC analysis of the total hydrocarbon fraction from female cockroaches showed it to be qualitatively (Fig. 5.3C) and quantitatively (Table 5.1) similar to hydrocarbons in oothecae and frass. From 50 adult female *P. americana*, 6.355 mg of 6,9-heptacosadiene was

collected. GC analysis of the purified 5 female equivalent sample by three temperature programs estimated its purity at 96.8, 98+, and 100%. Bioassay (n=4 bioassay replicates) indicated that ca. one OE of 6,9-heptacosadiene was 92.2% as active as an ootheca; subsequent bioassay of 0.5 ug elicited a response 78.9% as active as an ootheca (n=1 bioassay replicate).

Volatile 6,9-heptacosadiene from *P. americana* oothecae was effectively trapped on Super Q (Fig. 5.5B). Though the adsorbent was prepared for use by elution with 4-5 mls of hexane, some contaminants remained and were eluted with the hydrocarbons after volatile collection was complete (Fig. 5.5B & C (unlabeled peaks)). Identification of peak 3 in Fig. 5.5B was determined by several methods. First, via coinjection with alkane standards its Kovats retention index (KI) was 2666 (n=2 independent volatile collections); the KI of 6,9-heptacosadiene from *P. americana* oothecae was 2673. In addition, coinjection of known 6,9-heptacosadiene (Fig. 5.4D) and the volatile collection from oothecae (Fig. 5.5B) resulted in elution of a single peak at the retention time of the alkadiene. The injection was performed isothermally (60°C with a 2 min hold, then 5°C per min to 320°C with a 5 minute hold) and with the same temperature program reported earlier. It was also suspected that peaks 1 and 2 in Fig. 5.5B were pentacosane and 3-methylpentacosane, respectively, as compared with the reference chromatogram. The KI of 3-methylpentacosane from volatile collection was 2571, while the KI from ootheca-derived material was 2575. Pentacosane was identified in the volatile collection by comparison of its retention time with an authentic pentacosane standard, and its coelution with pentacosane standard. Fig. 5.5B represents 250.5 ootheca h equivalents injected, or

8.377±0.45 ng (n=2 determinations) 6,9-heptacosadiene quantified by coinjection with 0.1 ug hexacosane.

Discussion

Hydrocarbons account for the majority of cuticular lipids in P. americana; Gilby and Cox (1963) and Jackson (1972) estimated that 75%, and 85-95%, respectively, of cuticular lipids in P. americana are hydrocarbons. Baker et al. (1963) reported a bimodal distribution of hydrocarbons from P. americana cuticle and hemolymph, but only the first series of 5 peaks were identified. Hydrocarbon composition from the 2 sources was qualitatively identical and quantitatively similar. The vast majority (95% to 98%) of hydrocarbon is in the first series of peaks, containing 5 compounds of 25-29 carbons: n-pentacosane (12%), 3-methylpentacosane (20%), 6,9-heptacosadiene (65%), n-heptacosane, and n-nonacosane. Qualitative and quantitative hydrocarbon data from oothecae, frass, and adult female P. americana in the current study parallel those of earlier researchers (see Fig 5.3 and Table 5.1); the major hydrocarbon from all three sources was 6,9-heptacosadiene.

Because of the simplicity of its hydrocarbon composition, P. americana has been used as a model for hydrocarbon synthesis in insects. Nelson (1969) first demonstrated that hydrocarbons were synthesized in the epidermis (i.e. oenocytes) of P. americana; it is likely that a lipophorin is responsible for the transport of hydrocarbons to their final destination (Katase & Chino 1982). Jackson and Baker (1970) and Conrad and Jackson (1971) reported that injection of labelled linoleic acid (C18:2, at carbons 6 and 9) into adult P. americana resulted in incorporation of the label primarily into 6,9-heptacosadiene,

suggesting that linoleic acid is a precursor to this alkadiene. Dwyer et al. (1981) later documented that 6,9-heptacosadiene was in fact synthesized in P. americana by elongation of linoleic acid with acetate units, followed by decarboxylation to yield the alkadiene.

This study is the first evidence for kairomonal activity of an alkadiene, and the first biological activity of 6,9-heptacosadiene, though its existence has been known from P. americana since Baker et al. (1963) identified it; its identity has since been confirmed (Beatty & Gilby 1969, Jackson 1972). Other alkadienes, however, are biologically active; 7,11-heptacosadiene is an aphrodisiac found in the cuticular wax of female Drosophila melanogaster (Antony & Jallon 1982, Antony et al. 1985), and 6,9-nonadecadiene is a component of the sex pheromone of several Geometrid moth species (Szocs et al. 1984, McDonough et al. 1986). Bartelt et al. (1982) identified the sex pheromone blend of a sawfly, Pikonema alaskensis, as a series of 9,19-alkadienes (C28 to C39). Bartelt and Jones (1983) later discovered that some of the 9,19-alkadienes slowly air oxidized to aldehydes, and one prominent aldehyde, (Z)-10-nonadecenal, had primary pheromonal activity. The smallest alkadiene (C28) had the greatest amount of loss (47%) after a 24 h exposure to air. The kairomonal activity of 6,9-heptacosadiene may be an aldehyde product of its oxidation, but in our bioassays the alkadiene was not exposed to air until its bioassay, and then for a period of only 10 minutes. Additionally, the alkadiene was collected as a volatile on Super Q (Fig. 5.5B), an indication of its lack of oxidation.

Identification of a kairomone in the frass and cuticle of a host was first documented by Jones et al. (1971); 13-methylhentriacontane was identified in the frass, larvae, saliva, and hemolymph of H. zea as a host-seeking cue for its parasitoid M.

croceipes. When parasitoids contact this hydrocarbon, they intensely search the area by rubbing the substratum with their antennae. The location of the methyl group and the carbon chain length were very important to the activity of this kairomone, which was active at 50 ng. Other methylated hydrocarbons have been shown to have kairomonal properties; 13,17-dimethylnonatriacontane from moth scales was identified as effecting the host-seeking behavior in T. nubilale, an egg parasitoid of Ostrinia nubilalis (Shu et al. 1990).

The discovery of parasitoid-behavior modifying compounds is important not only from a basic biology standpoint, but has also been shown to have practical applications as well. Lewis et al. (1972) enhanced significantly the parasitism of Lepidoptera eggs by T. evanescens in Petri dishes, greenhouses, and cotton fields by pre-release application of a hexane-soluble host-location kairomone, later identified as tricosane, from moth scales. Eggs placed in locations treated with the extract suffered higher parasitism than control eggs (i.e. no kairomone). In a later study, Lewis et al. (1975) discovered that broadcast application of tricosane resulted in increased parasitism of sentinel and wild Lepidoptera eggs by wild and released Trichogramma spp. Unlike the inundative release of sex pheromone for mating disruption, large-scale application of a host-seeking stimulant did not serve as a confusant for the parasitoids; it stimulated host-searching, resulting in increased parasitism. The possibility of utilizing 6,9-heptacosadiene as a similar host-seeking stimulant for P. americana oothecae should be considered. The growing interest in biological and biorational (IGRs and pheromones) control of urban pests, fueled by a growing concern of the dangers of pesticide usage in the urban environment, will certainly

provide ample opportunity to investigate their chemical ecologies, and hopefully lead to the development of alternative control strategies to aid in their management.

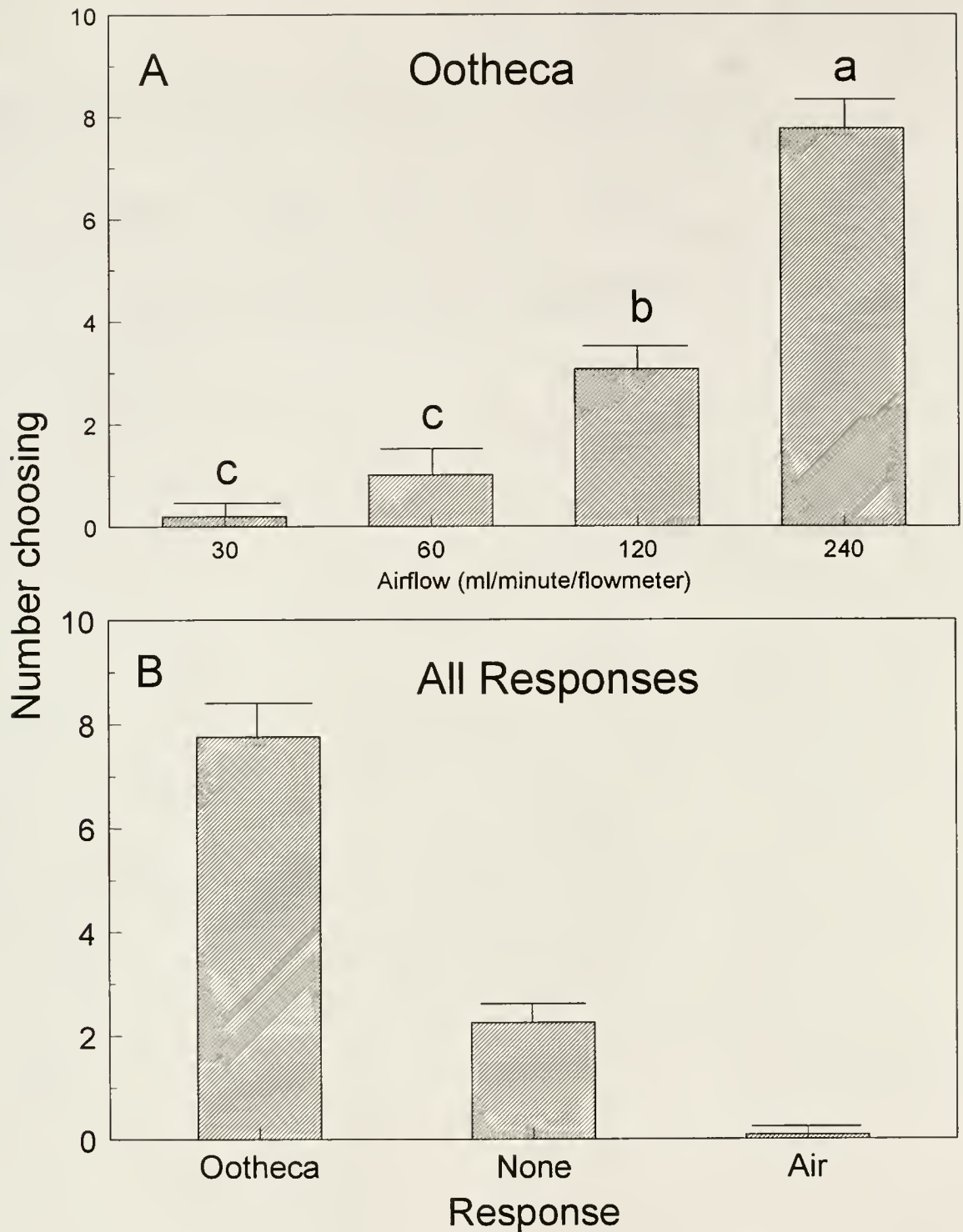


Fig. 5.1. Ten minute Y-tube bioassay response (mean $\pm$ SE) of 10, 1-d-old unfed *A. hagenowii* to (A) a <10-d-old *P. americana* ootheca at each of four airflow rates, and (B) all possible responses at the airflow rate of 240 ml/min/flowmeter. Bars separated by different letters are significantly different ($F=50.4$, $df=3,45$, $P<.0001$) (Tukey's Studentized range test).

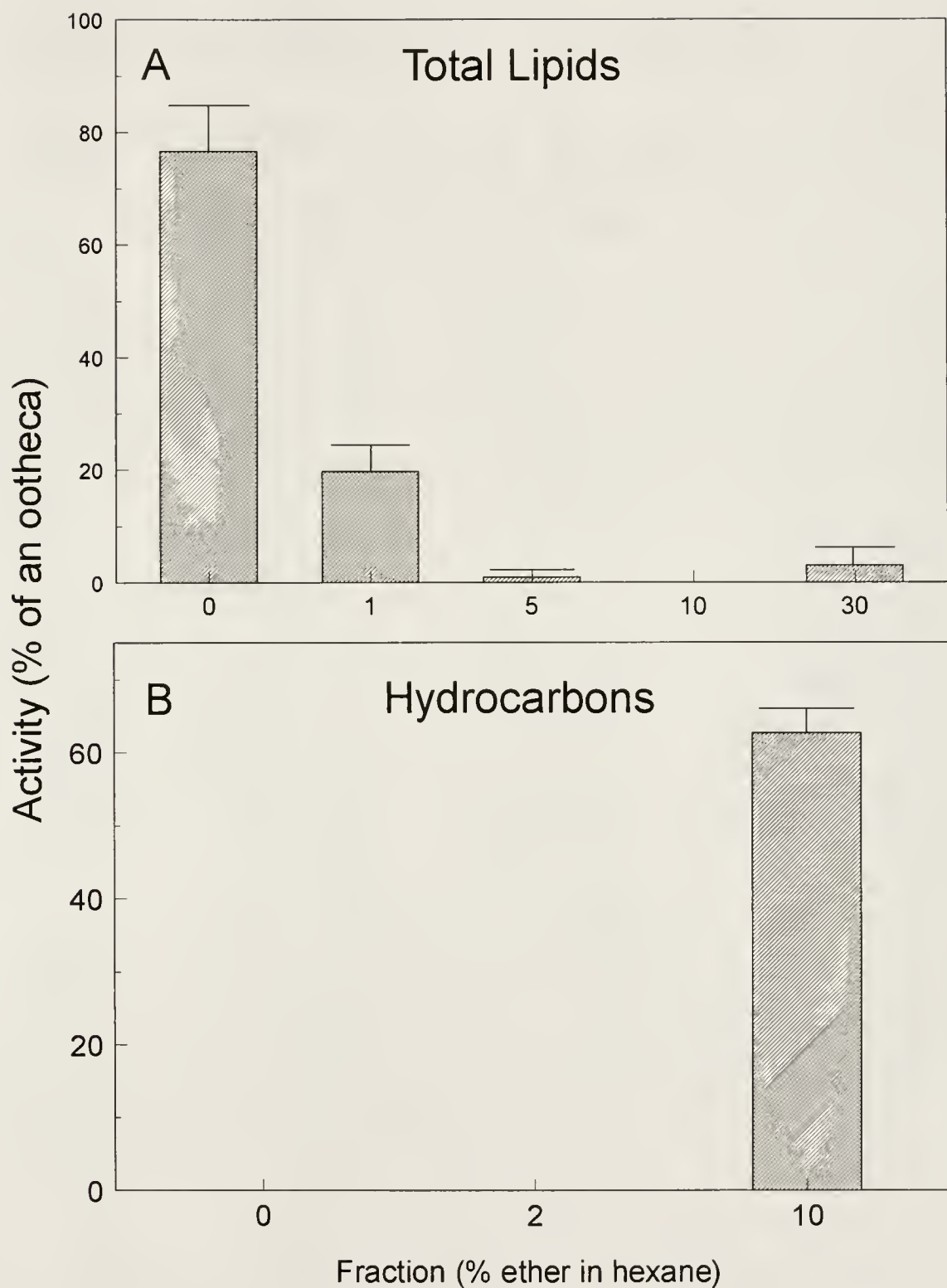


Fig. 5.2. Activity (mean $\pm$ SE) of one OE of (A) total lipids chromatographed on 70-230 mesh silica gel, and (B) hydrocarbons separated on 10% silver nitrate-impregnated silica gel.

Fig. 5.3. Gas chromatographic analysis of *P. americana* total hydrocarbon from (A) 1 to 7-d-old oothecae, (B) 1 to 5-d-old frass, and (C) adult females. Hydrocarbon identification after Baker et al. (1963). Peak 1: n-pentacosane; Peak 2: 3-methylpentacosane; Peak 3: 6,9-heptacosadiene; Peak 4: n-heptacosane; Peak 5: n-nonacosane. See Table 5.1 for corresponding peak area percentages. For each chromatogram, peak 3 is presented off scale to highlight smaller peaks. In chromatogram B, peak S is 0.1 ug n-hexacosane standard.

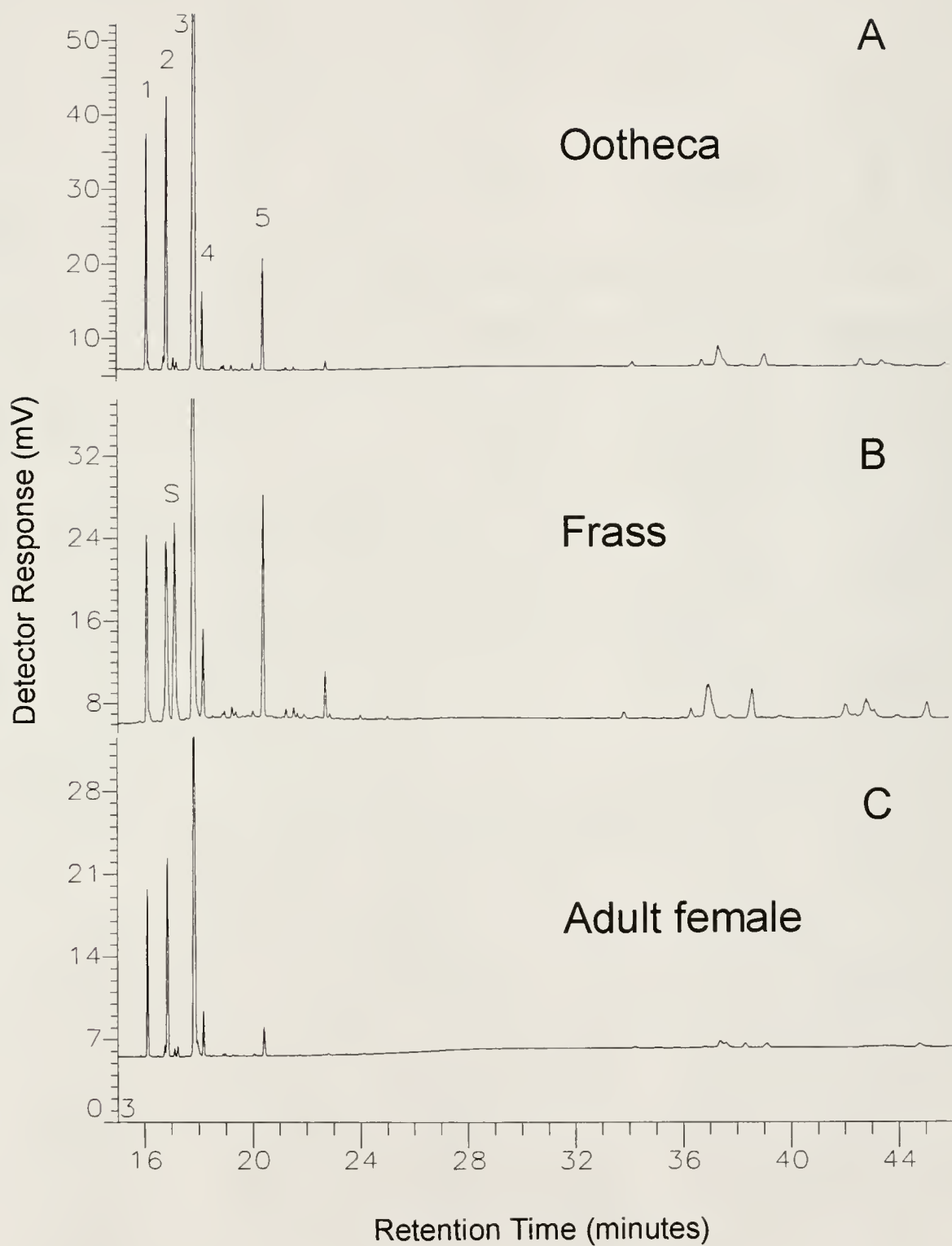


Table 5.1. Peak area percentage of hydrocarbons from *P. americana* oothecae, frass and adult female.

Hydrocarbon Source	N	% Peak Area (mean $\pm$ SE) ^a					% of total
		1	2	3	4	5	
Ootheca	5	12.4 $\pm$ 0.83	14.7 $\pm$ 0.61	60.0 $\pm$ 1.04	3.4 $\pm$ 0.16	5.5 $\pm$ 0.23	96.0 $\pm$ 1.71
Frass	4	11.5 $\pm$ 1.30	12.9 $\pm$ 0.58	45.9 $\pm$ 1.10	4.8 $\pm$ 0.45	11.9 $\pm$ 2.33	87.0 $\pm$ 3.45
Female	4	12.9 $\pm$ 0.23	15.2 $\pm$ 0.18	62.7 $\pm$ 0.91	2.9 $\pm$ 0.14	3.0 $\pm$ 0.06	96.7 $\pm$ 0.59

^a 1=n-pentacosane, 2=3-methylpentacosane, 3=6,9-heptacosadiene, 4=n-heptacosane, 5=n-nonacosane.

Fig. 5.4. Gas chromatographic analysis of (A) total hydrocarbon from 1 to 7-d-old *P. americana* oothecae (as a reference chromatogram), and ootheca hydrocarbons which were separated on 10% AgNO₃-impregnated silica gel by elution with (B) 0, (C) 2, and (D) 10% ether in hexane. 0.2 ootheca equivalents were injected for each of the 0, 2, and 10% fractions.

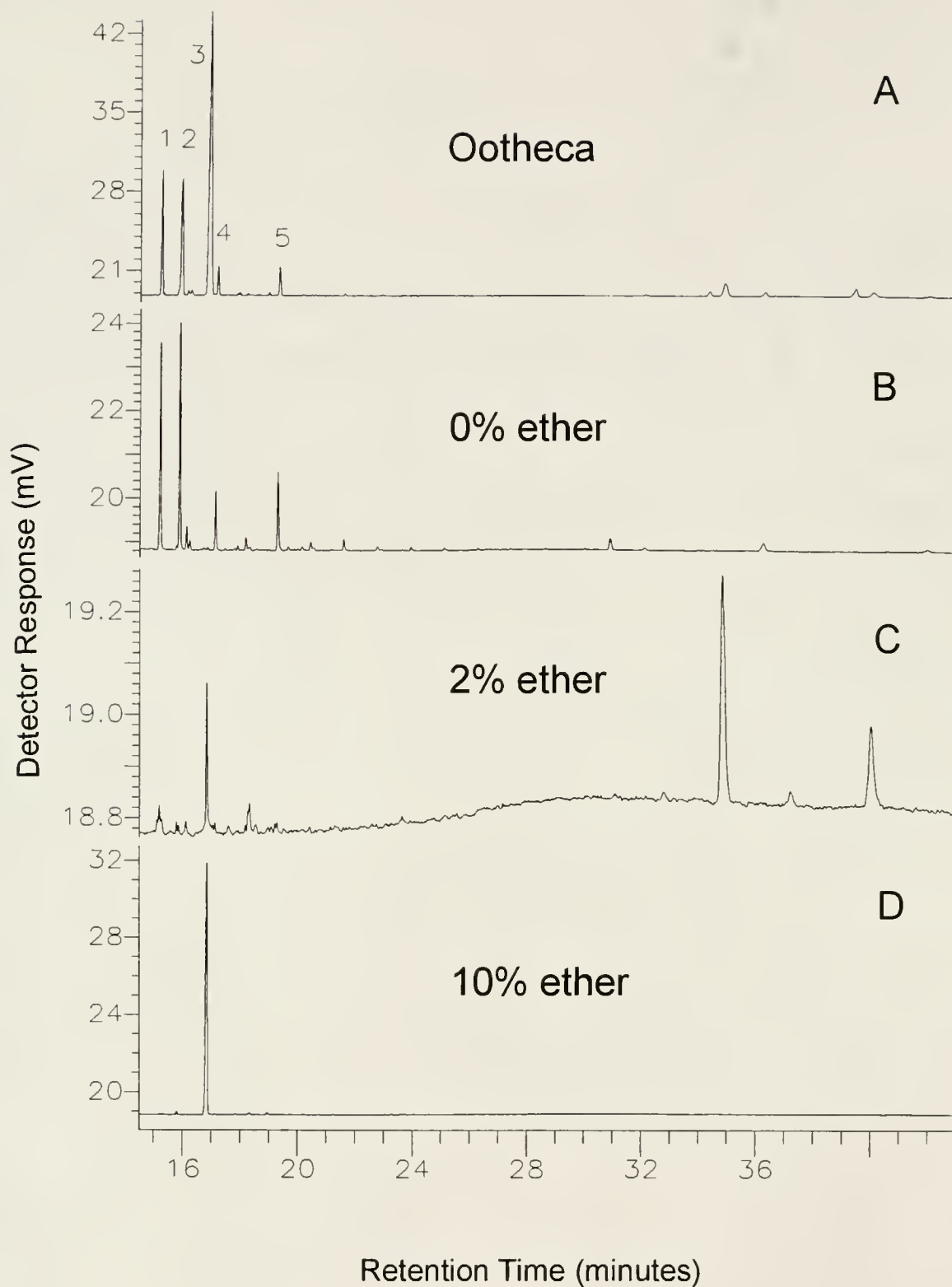
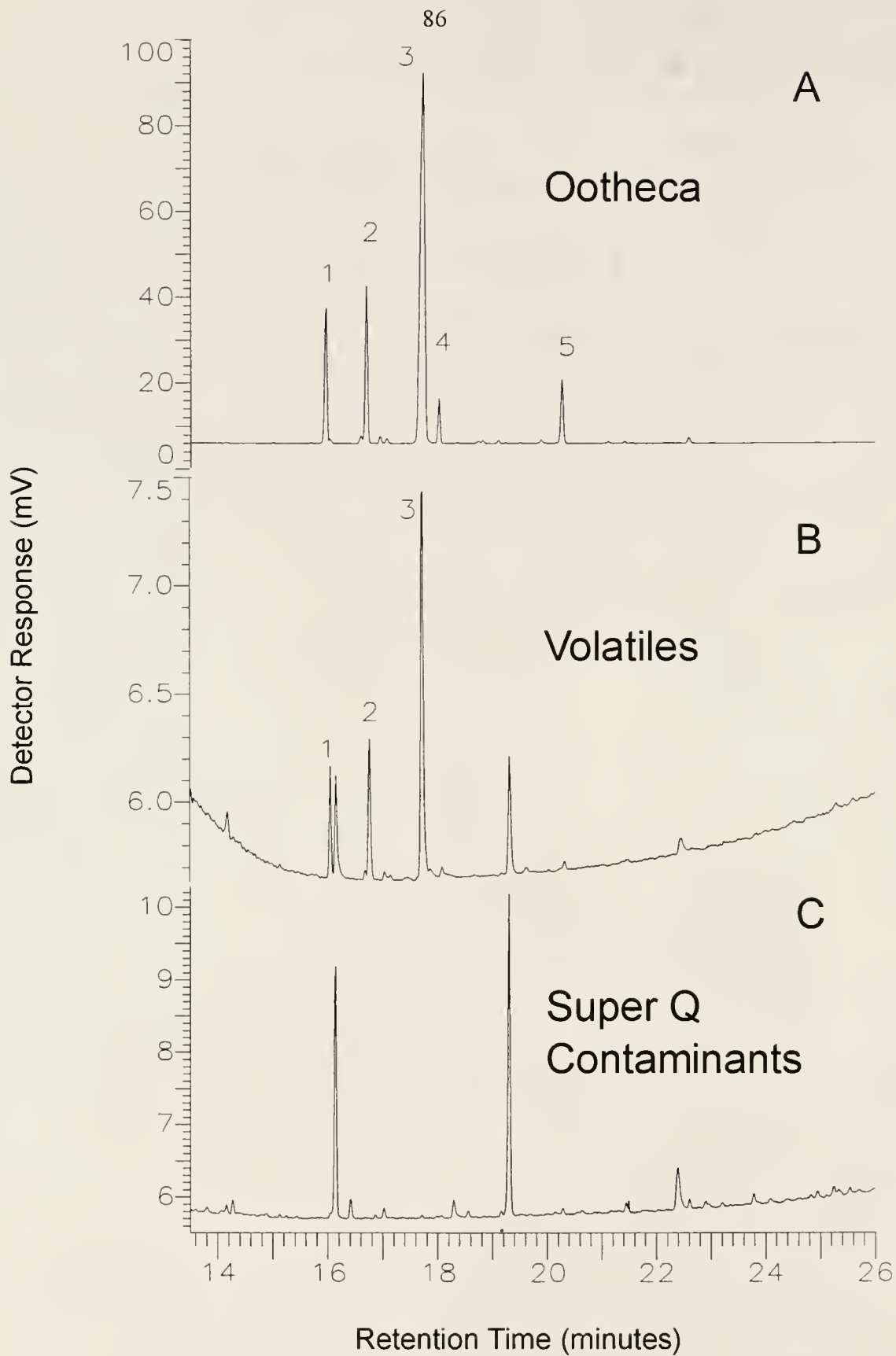


Fig. 5.5. Gas chromatographic analysis of (A) *P. americana* ootheca total hydrocarbon (as a reference chromatogram), (B) volatile hydrocarbons from <10-d-old oothecae, and (C) contaminants from Super Q volatile adsorbent. Peak 1=n-pentacosane, 2=3-methylpentacosane, 3=6,9-heptacosadiene, 4=n-heptacosane, 5=n-nonacosane.



REFERENCES CITED

- Ali, M. & W. Islam. 1983. Morphology of Tetrastichus hagenowii Chalcidoidea Tetrastichidae a parasite on the oothecae of cockroach. Bangladesh J. Zool. 11(2): 79-86.
- Antony, C., T. L. Davis, D. A. Carlson, J. W. Pechine & J. M. Jallon. 1985. Compared behavioral responses of male Drosophila melanogaster (Canton S) to natural and synthetic aphrodisiacs. J. Chem. Ecol. 11(12): 1617-1629.
- Antony, C. & J. M. Jallon. 1982. The chemical basis for sex recognition in Drosophila melanogaster. J. Ins. Physiol. 28: 873-880.
- Appel, A. G. & M. K. Rust. 1985. Outdoor activity and distribution of the smokybrown cockroach, Periplaneta fuliginosa (Dictyoptera: Blattidae). Environ. Entomol. 14: 669-673.
- Arthur, A. P. 1981. Host acceptance by parasitoids, pp. 97-120. In D. A. Nordlund, R. L. Jones & W. J. Lewis [eds.], Semiochemicals: Their role in pest control. Wiley & Sons, New York, New York.
- Auger, J., C. LeComte, J. Paris & E. Thibout. 1989. Identification of leek-moth and diamondback-moth volatiles that stimulate parasitoid, Diadromus pulchellus. J. Chem. Ecol. 15(4): 1391-1398.
- Baehrecke, E. H., S. B. Vinson & H. J. Williams. 1990. Foraging behavior of Camponotus sonorensis in response to Heliothis virescens and cotton plants. Entomol. Exp. Appl. 55: 47-57.
- Baker, G. L., H. E. Vroman & J. Padmore. 1963. Hydrocarbons of the American cockroach. Biochem. Biophys. Res. Commun. 13: 360-365.
- Barlin, M. R., S. B. Vinson & G. L. Piper. 1981. Ultrastructure of the antennal sensilla of the cockroach-egg parasitoid, Tetrastichus hagenowii (Hymenoptera: Eulophidae). J. Morph. 168: 97-108.

- Bartelt, R. J. & R. L. Jones. 1983. (Z)-10-nonadecenal: A pheromonally active air oxidation product of (Z,Z)-9,19-dienes in yellowheaded spruce sawfly. *J. Chem. Ecol.* 9(9): 1333-1341.
- Bartelt, R. J., R. L. Jones & H. M. Kulman. 1982. Hydrocarbon components of the yellowheaded spruce sawfly sex pheromone: A series of (Z,Z)-9,19-dienes. *J. Chem. Ecol.* 8(1): 95-114.
- Beatty, I. M. & A. R. Gilby. 1969. The major hydrocarbon of a cockroach cuticular wax. *Naturwissenschaften* 25: 373.
- Bouchard, Y. & C. Cloutier. 1984. Honeydew as a source of host-searching kairomones for the aphid parasitoid Aphidius nigripes (Hymenoptera: Aphidiidae). *Can. J. Zool.* 62: 1513-1520.
- Bouchard, Y. & C. Cloutier. 1985. Role of olfaction in host finding by aphid parasitoid Aphidius nigripes (Hymenoptera: Aphidiidae). *J. Chem. Ecol.* 11(6): 801-808.
- Brenner, R. J. 1988. Focality and mobility of some peridomestic cockroaches in Florida (Dictyoptera: Blattaria). *Ann. Entomol. Soc. Am.* 81(4): 581-592.
- Brenner, R. J. & R. R. Pierce. 1991. Seasonality of peridomestic cockroaches (Blattoidea: Blattidae): mobility, winter reduction, and effect of traps and baits. *J. Econ. Entomol.* 84(6): 1735-1745.
- Brill, J. H. & W. Bertsch. 1985. An investigation of sampling methods for the analysis of cuticular hydrocarbons. *J. Entomol. Sci.* 20(4): 435-443.
- Burks, B. D. 1943. The North American parasitic wasps of the genus Tetrastichus- a contribution to biological control of insect pests. *Proceedings of the United States National Museum* 93(3170): 505-608.
- Cameron, E. 1955. On the parasites and predators of the cockroach. I. Tetrastichus hagenowii (Ratz.). *Bull. Entomol. Res.* 46: 137-147.
- Carde, R. T. & H. Lee. 1989. Effect of experience on the responses of the parasitoid Brachymeria intermedia (Hymenoptera: Chalcididae) to its host, Lymantria dispar (Lepidoptera: Lymantriidae), and to kairomone. *Ann. Entomol. Soc. Am.* 82(5): 653-657.
- Carlson, D. A. & M. W. Service. 1980. Identification of mosquitoes of Anopheles gambiae species complex A & B by analysis of cuticular components. *Science* 207: 1089-1091.

- Carroll, K. K. 1961. Separation of lipid classes by chromatography on florisil. *J. Lipid Research*. 2(2): 135-141.
- Cave, R. D., M. J. Gaylor & J. T. Bradley. 1987. Host handling and recognition by Telenomus reynoldsi (Hymenoptera: Scelionidae), an egg parasitoid of Geocoris spp. (Heteroptera: Lygaeidae). *Ann. Entomol. Soc. Am.* 80: 217-223.
- Ceianu, C. 1986. Two parasites of Blatta orientalis L. oothecae: Tetrastichus hagenowii (Ratzeburg) (Hymenoptera: Eulophidae) and Prosevania fuscipes (Illiger) (Hymenoptera: Evaniidae). *Arch. Roum. Pathol. Exp. Microbiol.*, Bucharest. 45(2): 161-167.
- Chiri, A. A. & E. F. Legner. 1986. Response of three Chelonus (Hymenoptera: Braconidae) species to kairomones in scales of six Lepidoptera. *Can. Ent.* 118: 329-333.
- Clement, S. L., W. L. Rubini & D. A. McCartney. 1986. Larviposition response of Bonnetia compta (Dipt.: Tachinidae) to a kairomone of Agrotis ipsilon (Lep.: Noctuidae). *Entomophaga* 31(1): 277-284.
- Conover, W. J., M. E. Johnson & M. M. Johnson. 1981. A comparative study of tests for homogeneity of variances, with applications to the outer continental shelf bidding data. *Technometrics* 23(4): 351-361.
- Conrad, C. W. & L. L. Jackson. 1971. Hydrocarbon biosynthesis in Periplaneta americana. *J. Ins. Physiol.* 17: 1907-1916.
- Cornwell, P. B. 1968. The cockroach, vol. 1. Hutchinson, London.
- Dmoch, J., W. J. Lewis, P. B. Martin & D. A. Nordlund. 1985. Role of host-produced stimuli and learning in host selection behavior of Cotesia (= Apanteles) marginiventris (Cresson). *J. Chem. Ecol.* 11(4): 453-463.
- Drost, Y. C., W. J. Lewis, P. O. Zanen & M. A. Keller. 1986. Beneficial arthropod behavior mediated by airborne semiochemicals. I. Flight behavior and influence of preflight handling of Microplitis croceipes (Cresson). *J. Chem. Ecol.* 12(6): 1247-1262.
- Dwyer, L. A., M. de Renobales & G. L. Blomquist. 1981. Biosynthesis of (Z,Z)-6,9-heptacosadiene in the American cockroach. *Lipids* 16(11): 810-814.

- Edmunds, L. R. 1955. Biological notes on Tetrastichus hagenowii (Ratzeburg), a chalcidoid parasite of cockroach eggs (Hymenoptera: Eulophidae; Orthoptera: Blattidae). *Ann. Entomol. Soc. Am.* 48: 210-213.
- Eller, F. J., J. H. Tumlinson & W. J. Lewis. 1988. Beneficial arthropod behavior mediated by airborne semiochemicals: Source of volatiles mediating the host-location flight behavior of Microplitis croceipes (Cresson) (Hymenoptera: Braconidae), a parasitoid of Heliothis zea (Boddie) (Lepidoptera: Noctuidae). *Environ. Entomol.* 17(4): 745-753.
- Elzen, G. W., H. J. Williams & S. B. Vinson. 1983. Response by the parasitoid Campoletis sonorensis (Hymenoptera: Ichneumonidae) to chemicals (synomones) in plants: Implications for host habitat location. *Environ. Entomol.* 12:1873-1877.
- Elzen, G. W., H. J. Williams & S. B. Vinson. 1984. Isolation and identification of cotton synomones mediating searching behavior of parasitoid Campoletis sonorensis. *J. Chem. Ecol.* 10(8): 1251-1264.
- Elzen, G. W., H. J. Williams & S. B. Vinson. 1986. Wind tunnel flight responses by hymenopterous parasitoid Campoletis sonorensis to cotton cultivars and lines. *Entomol. Exp. Appl.* 42: 285-289.
- Fleet, R. R. & G. W. Frankie. 1975. Behavioral and ecological characteristics of a eulophid egg parasite of two species of domiciliary cockroaches. *Environ. Entomol.* 4(2): 282-284.
- Fleet, R. R., G. L. Piper & G. W. Frankie. 1978. Studies on the population ecology of the smokybrown cockroach, Periplaneta fuliginosa, in a Texas outdoor urban environment. *Environ. Entomol.* 7: 807-814.
- Fukushima, J., Y. Kuwahara & T. Suzuki. 1989. Isolation and identification of a kairomone responsible for the stinging behavior of Bracon hebetor Say (Hymenoptera: Braconidae) from frass of the Almond moth Cadra cautella Walker. *Agric. Biol. Chem.* 53(11): 3057-3059.
- Gilby, A. R. & M. E. Cox. 1963. The cuticular lipids of the cockroach, Periplaneta americana (L.). *J. Ins. Physiol.* 9: 671-681.
- Greany, P. D., S. B. Vinson & W. J. Lewis. 1984. Insect parasitoids: Finding new opportunities for biological control. *Bioscience* 34(11): 690-696.

- Gueldner, R. C., D. A. Nordlund, W. J. Lewis, J. E. Thean & D. M. Wilson. 1984. Kairomones and their use for management of entomophagous insects. XV. Identification of several acids in scales of Heliothis zea moths and comments on their possible role as kairomones for Trichogramma pretiosum. J. Chem. Ecol. 10(2): 245-251.
- Hagenbuch, B. E., R. S. Patterson & P. G. Koehler. 1989. Biological control of the American cockroach (Orthoptera: Blattidae) with inundative releases of Tetrastichus hagenowii (Hymenoptera: Eulophidae). J. Econ. Entomol. 82(1): 90-94.
- Hagenbuch, B. E., R. S. Patterson, P. G. Koehler & R. J. Brenner. 1988. Mass production of the cockroach oothecal parasitoid, Tetrastichus hagenowii (Hymenoptera: Eulophidae), and its host, the American cockroach (Orthoptera: Blattidae). J. Econ. Entomol. 81(2): 531-535.
- Hagvar, E. B. & T. Hofsvang. 1989. Effect of honeydew and hosts on plant colonization by the aphid parasitoid Ephedrus cerasicola. Entomophaga 34(4): 495-501.
- Harlan, H. J. & R. D. Kramer. 1981. Limited host specificity in Tetrastichus hagenowii (Ratzeburg). J. Georgia Entomol. Soc. 16(1): 67-70.
- Heitmans, W. R. B., P. Haccou & J. J. M. van Alphen. 1992. Egg supply, clutch size and survival probability in Aprostocetus hagenowii (Ratz) (Hymenoptera: Eulophidae), a gregarious parasitoid of cockroach oothecae. Proc. Exper. & Appl. Entomol. 3: 62-69.
- Henson, R. D., S. B. Vinson & C. S. Barfield. 1977. Ovipositional behavior of Bracon mellitor Say, a parasitoid of the boll weevil (Anthonomus grandis Boheman). III. Isolation and identification of natural releasers of ovipositor probing. J. Chem. Ecol. 3(2): 151-158.
- Herard, F., M. A. Keller, W. J. Lewis & J. H. Tumlinson. 1988a. Beneficial arthropod behavior mediated by airborne semiochemicals. III. Influence of age and experience on flight chamber responses of Microplitis demolitor Wilkinson. J. Chem. Ecol. 14(7): 1583-1596.
- Herard, F., M. A. Keller, W. J. Lewis & J. H. Tumlinson. 1988b. Beneficial arthropod behavior mediated by airborne semiochemicals. IV. Influence of host diet on host-oriented flight chamber responses of Microplitis demolitor Wilkinson. J. Chem. Ecol. 14(7): 1597-1606.

- Hochberg, Y. & A. C. Tamhane. 1987. Multiple Comparison Procedures. Wiley & Sons, New York, New York.
- Howard, R. W. & P. W. Flinn. 1990. Larval trails of Cryptolestes ferrugineus (Coleoptera: Cucujidae) as kairomonal host-finding cues for the parasitoid Cephalonomia waterstoni (Hymenoptera: Bethyridae). Ann. Entomol. Soc. Am. 83(2): 239-245.
- Jackson, L. 1972. Cuticular lipids of insects-IV. Hydrocarbons of the cockroaches Periplaneta japonica and Periplaneta americana compared to other cockroach hydrocarbons. Comp. Biochem. Physiol. 41B: 331-336.
- Jackson, L. L. & G. L. Baker. 1970. Cuticular lipids of insects. Lipids 5(2): 239-246.
- Jie, L. & N. Wenqing. 1984. Bionomics of Tetrastichus hagenowii parasitizing in the oothecae of Periplaneta americana. Acta Entomol. Sin. 27(4): 406-409.
- Jones, R. L., W. J. Lewis, M. Beroza, B. A. Bierl & A. N. Sparks. 1973. Host-seeking stimulants (kairomones) for the egg parasite, Trichogramma evanescens. Environ. Entomol. 2(4): 593-596.
- Jones, R. L., W. J. Lewis, M. C. Bowman, M. Beroza & B. A. Bierl. 1971. Host-seeking stimulant for parasite of corn earworm: Isolation, identification, and synthesis. Science 173: 842-843.
- Kanayama, A., E. Yoshida & T. Honma. 1975. The parasitism by Tetrastichus hagenowii (Ratzeburg) on oothecae of the smoky brown cockroach, Periplaneta fuliginosa (Serv.) collected in Shizuoka city. Jpn. J. Sanit. Zool. 27(2): 157-162.
- Katase, H. & H. Chino. 1982. Transport of hydrocarbons by the lipophorin of insect hemolymph. Biochim. Biophys. Acta 710: 341-348.
- Keller, M. A. 1990. Responses of the parasitoid Cotesia rubecula to its host Pieris rapae in a flight tunnel. Entomol. Exp. Appl. 57: 243-249.
- Kumarasinghe, N. C. & J. P. Edirisinghe. 1987. Oothecal parasites of Periplaneta americana: Parasitization and development in relation to host age. Insect Sci. Applic. 8(2): 225-228.
- Kumarasinghe, N. C. & J. P. Edirisinghe. 1991. A preliminary survey of domiciliary cockroaches and their oothecal parasites in Sri Lanka. Vidyodaya J. Sci. 3(2): 35-43.

- LeBeck, L. M. 1991. A review of the Hymenopterous natural enemies of cockroaches with emphasis on biological control. *Entomophaga* 36(3): 335-352.
- Leonard, D. E., B. A. Bierl & M. Beroza. 1975. Gypsy moth kairomones influencing behavior of the parasitoids Brachymeria intermedia and Apanteles melanoscelus. *Environ. Entomol.* 4(6): 929-930.
- Leonard, D. E., Z. Wu & D. N. Ferro. 1987. Responses of parasite Edovum puttleri to kairomone from eggs of Colorado potato beetle, Leptinotarsa decemlineata. *J. Chem. Ecol.* 13(2): 335-344.
- Levene, H. 1960. Robust tests for equality of variances, pp. 278-292. In I. Olkin [ed.], *Contributions to Probability and Statistics*. Stanford University Press, Palo Alto.
- Lewis, W. J. & R. L. Jones. 1971. Substance that stimulates host-seeking by Microplitis croceipes (Hymenoptera: Braconidae), a parasite of Heliothis species. *Ann. Entomol. Soc. Am.* 64(2): 471-473.
- Lewis, W. J., R. L. Jones, D. A. Nordlund & A. N. Sparks. 1975. Kairomones and their use for management of entomophagous insects: I. Evaluation for increasing rates of parasitization by Trichogramma spp. in the field. *J. Chem. Ecol.* 1(3): 343-347.
- Lewis, W. J., R. L. Jones & A. N. Sparks. 1972. A host seeking stimulant for the egg parasite Trichogramma evanescens: Its source and a demonstration of its laboratory and field activity. *Ann. Entomol. Soc. Am.* 65(5): 1087-1089.
- Lewis, W. J. & D. A. Nordlund. 1984. Semiochemicals influencing fall armyworm parasitoid behavior: Implications for behavioral manipulation. *Fla. Entomol.* 67(3): 343-349.
- Lewis, W. J. & J. H. Tumlinson. 1988. Host detection by chemically mediated associative learning in a parasitic wasp. *Nature* 331: 257-259.
- Lewis, W. J., J. H. Tumlinson & S. Krasnoff. 1991. Chemically mediated associative learning: An important function in the foraging behavior of Microplitis croceipes (Cresson). *J. Chem. Ecol.* 17(7): 1309-1325.
- Li, L. Y. 1990. Mass production of Trichogramma spp. and Anastatus japonicus Ashmead with artificial diets in China. FFTC-NARC International Seminar on 'The use of Parasitoids and Predators to Control Agricultural Pests', Tukuho Science City, Ibaraki-Ken, 305, Japan, October 2-7, 1989.

- Ma, R. Z., P. D. Swedenborg & R. L. Jones. 1992. Host-seeking behavior of Eriborus terebrans (Hymenoptera: Ichneumonidae) toward the european corn borer and the role of chemical stimuli. *Ann. Entomol. Soc. Am.* 85(1): 72-79.
- Mack, G. A. & J. H. Skillings. 1980. A Friedman-type rank test for main effects in a two-factor ANOVA. *J. of the Amer. Stat'l. Assn.* 75: 947-951.
- McAuslane, H. J., S. B. Vinson & H. J. Williams. 1990. Effect of host diet on flight behavior of the parasitoid Campoletis sonorensis (Hymenoptera: Ichneumonidae). *J. Entomol. Sci.* 25(4): 562-570.
- McAuslane, H. J., S. B. Vinson & H. J. Williams. 1991a. Stimuli influencing host microhabitat location in the parasitoid Campoletis sonorensis. *Entomol. Exp. Appl.* 58: 267-277.
- McAuslane, H. J., S. B. Vinson & H. J. Williams. 1991b. Influence of adult experience on host microhabitat location by the generalist parasitoid, Campoletis sonorensis (Hymenoptera: Ichneumonidae). *J. Ins. Beh.* 4(1): 101-113.
- McDonough, L. M., J. B. Bailey, M. P. Hoffmann, B. A. Leonhardt, D. F. Brown, C. L. Smithhisler & K. Olsen. 1986. Sabulodes caberata Guenee (Lepidoptera: Geometridae) components of its sex pheromone gland. *J. Chem. Ecol.* 12(12): 2107-2116.
- Morgan, P. B., B. J. Smittle & R. S. Patterson. 1986. Use of irradiated pupae to mass culture the microhymenopterous pupal parasitoid Spalangia endius Walker (Hymenoptera: Pteromalidae). I. Musca domestica L. (Diptera: Muscidae). *J. Entomol. Sci.* 21(3): 222-227.
- Mudd, A. & S. A. Corbet. 1982. Response of the Ichneumonid parasite Nemeritis canescens to kairomones from the flour moth, Ephestia kuehniella. *J. Chem. Ecol.* 8(5): 843-850.
- Mudd, A. J., H. H. Walters & S. A. Corbet. 1984. Relative kairomonal activities of 2-acetylcyclohexane-1,3-diones in eliciting oviposition behavior from the parasite Nemeritis canescens (Grav.). *J. Chem. Ecol.* 10(11): 1597-1601.
- Muzaffar, N. & C. Inayatullah. 1986. Role of salivary glands of Pyralid borer larvae in host selection by Apanteles flavipes (Cameron) (Hym: Braconidae). *J. Agric. Res.* 24(3): 203-205.

- Narasimham, A. U. 1984. Comparative studies on Tetrastichus hagenowii (Ratzeburg) and T. asthenogmus (Waterston), two primary parasites of cockroach oothecae, and on their hyperparasite Tetrastichus sp. (T. miser (Nees) group) (Hymenoptera: Eulophidae). Bull. Ent. Res. 74: 175-189.
- Narasimham, A. U. & T. Sankaran. 1979. Domiciliary cockroaches and their oothecal parasites in India. Entomophaga 24(3): 273-279.
- Nelson, D. R. 1969. Hydrocarbon synthesis in the American cockroach. Nature 221: 854-855.
- Nemoto, T., Y. Kuwahara & T. Suzuki. 1987a. Interspecific difference in Venturia kairomones in larval feces of four stored Phycitid moths. Appl. Ent. Zool. 22(4): 553-559.
- Nemoto, T., M. Shibuya, Y. Kuwahara & T. Suzuki. 1987b. New 2-acylcyclohexane-1,3-diones: Kairomone components against a parasitic wasp, Venturia canescens, from feces of the Almond moth, Cadra cautella, and the Indian Meal moth, Plodia interpunctella. Agric. Biol. Chem. 51(7): 1805-1810.
- Noldus, L. P. J. J. 1988. Response of the egg parasitoid Trichogramma pretiosum to the sex pheromone of its host Heliothis zea. Entomol. Exp. Appl. 48: 293-300.
- Noldus, L. P. J. J. & J. C. van Lenteren. 1985a. Kairomones for the egg parasite Trichogramma evanescens Westwood. I. Effect of substances released by two of its hosts, Pieris brassicae L. and Mamestra brassicae L. J. Chem. Ecol. 11: 781-791.
- Noldus, L. P. J. J. & J. C. van Lenteren. 1985b. Kairomones for the egg parasite Trichogramma evanescens Westwood. II. Effect of contact chemicals produced by two of its hosts, Pieris brassicae L. and Pieris rapae. J. Chem. Ecol. 11: 793-800.
- Noldus, L. P. J. J., J. C. van Lenteren & W. J. Lewis. 1991. How Trichogramma parasitoids use moth sex pheromones as kairomones: orientation behavior in a wind tunnel. Physiol. Entomol. 16: 313-327.
- Nordlund, D. A., R. L. Jones & W. J. Lewis. 1981. Semiochemicals: Their Role in Pest Control. Wiley & Sons, New York, New York.
- Nordlund, D. A. & W. J. Lewis. 1976. Terminology of chemical releasing stimuli in intraspecific and interspecific interactions. J. Chem. Ecol. 2(2): 211-220.

- Nordlund, D. A. & W. J. Lewis. 1985. Response of females of the braconid parasitoid Microplitis demolitor to frass of larvae of the noctuids, Heliothis zea and Trichoplusia ni and to 13-methylhentriacontane. *Entomol. Exp. Appl.* 38: 109-112.
- Nordlund, D. A., W. J. Lewis & R. C. Gueldner. 1983. Kairomones and their use for management of entomophagous insects. XIV. Response of Telenomus remus to abdominal tips of Spodoptera frugiperda, (Z)-9-tetradecene-1-ol acetate and (Z)-9-dodecene-1-ol acetate. *J. Chem. Ecol.* 9(6): 695-701.
- Nordlund, D. A., W. J. Lewis, R. L. Jones & H. R. Gross, Jr. 1976. Kairomones and their use for management of entomophagous insects. IV. Effect of kairomones on productivity and longevity of Trichogramma pretiosum Riley (Hymenoptera: Trichogrammatidae). *J. Chem. Ecol.* 2(1): 67-72.
- Nordlund, D. A., W. J. Lewis, R. L. Jones, H. R. Gross & K. S. Hagen. 1977a. Kairomones and their use for management of entomophagous insects. VI. An examination of the kairomones for the predator Chrysopa carnea Stephens at the oviposition sites of Heliothis zea (Boddie). *J. Chem. Ecol.* 3(5): 507-511.
- Nordlund, D. A., W. J. Lewis, J. W. Todd & R. B. Chalfant. 1977b. Kairomones and their use for management of entomophagous insects. VII. The involvement of various stimuli in the differential response of Trichogramma pretiosum Riley to two suitable hosts. *J. Chem. Ecol.* 3(5): 513-518.
- Nordlund, D. A. & C. E. Sauls. 1981. Kairomones and their use for management of entomophagous insects. XI. Effect of host plants on kairomonal activity of frass from Heliothis zea larvae for the parasitoid Microplitis croceipes. *J. Chem. Ecol.* 7(6): 1057-1061.
- Nordlund, D. A., M. R. Strand, W. J. Lewis & S. B. Vinson. 1987. Role of kairomones from host accessory gland secretion in host recognition by Telenomus remus and Trichogramma pretiosum, with partial characterization. *Entomol. Exp. Appl.* 44: 37-43.
- Ott, L. 1988. *An Introduction to Statistical Methods and Data Analysis*, 3rd ed. PWS-Kent, Boston, Massachusetts.
- Patterson, R. S., B. E. Hagenbuch, P. G. Koehler & R. J. Brenner. 1988. Efficiency of Tetrastichus hagenowii (Hymenoptera: Eulophidae) to control the American cockroach, Periplaneta americana (Orthoptera: Blattellidae). *Advances in Parasitic Hymenoptera Research* 433-443.

- Pawson, B. M. & R. E. Gold. 1993. Evaluating different release strategies for the control of American cockroaches using the egg parasitoid, Aprostocetus hagenowii, pp. 407-413. In Proceedings, First International Conference on Insect Pests in the Urban Environment, 30 June-3 July 1993. BPCC Wheatons Ltd., Exeter.
- Piper, G. L. & G. W. Frankie. 1978. Integrated management of urban cockroach populations, pp. 249-266. In G. W. Frankie & C. S. Koehler [eds.], Perspectives in Urban Entomology. Academic Press, New York, New York.
- Piper, G. L., G. W. Frankie & J. Loehr. 1978. Incidence of cockroach egg parasitoids in urban environments in Texas and Louisiana. *Environ. Entomol.* 7(2): 289-293.
- Prokopy, R. J. & R. P. Webster. 1978. Oviposition-detering pheromone of Rhagoletis pomonella: A kairomone for its parasitoid Opius lectus. *J. Chem. Ecol.* 4(4): 481-494.
- Renou, M., N. Hawlitzky, A. Berthier, C. Malosse & F. Ramiandrasoa. 1989. Evidences for kairomones for female Trichogramma maidis in the eggs of the european corn borer, Ostrinia nubilalis. *Entomophaga* 34(4): 569-580.
- Roth, L. M. & E. R. Willis. 1954. The biology of the cockroach egg parasite, Tetrastichus hagenowii (Hymenoptera: Eulophidae). *Trans. Am. Entomol. Soc.* 80: 53-72.
- Roth, L. M. & E. R. Willis. 1960. Biotic associations of cockroaches. *Smithsonian Misc. Coll.* 141.
- SAS Institute Inc. SAS/STAT User's Guide, Release 6.03 Edition. Cary, North Carolina: SAS Institute, Inc., 1988. 1028 pp.
- Shu, S. & R. L. Jones. 1989. Kinetic effects of a kairomone in moth scales of the european corn borer on Trichogramma nubilale Ertle & Davis (Hymenoptera: Trichogrammatidae). *J. Ins. Beh.* 2(1): 123-131.
- Shu, S., P. D. Swedenborg & R. L. Jones. 1990. A kairomone for Trichogramma nubilale (Hymenoptera: Trichogrammatidae): Isolation, identification and synthesis. *J. Chem. Ecol.* 16(2): 521-529.
- Skillings, J. H. & G. A. Mack. 1981. On the use of a Friedman-type statistic in balanced and unbalanced block designs. *Technometrics* 23: 171-177.
- Strand, M. R. & S. B. Vinson. 1983. Factors affecting host recognition and acceptance in the egg parasitoid Telenomus heliothidis (Hymenoptera: Scelionidae). *Environ. Entomol.* 12(4): 1114-1119.

- Szocs, G., M. Toth, H. Bestmann & O. Vostrowsky. 1984. A two-component sex attractant for males of the Geometrid moth Alsophila quadripunctata. Entomol. Exp. Appl. 36: 287-291.
- Takabayashi, J. & S. Takahashi. 1986. Effect of kairomones in the host searching behavior of Apanteles kariyai Watanabe (Hymenoptera: Braconidae), a parasitoid of the common armyworm, Pseudaletia separata Walker (Lepidoptera: Noctuidae). II. Isolation and identification of arrestants produced by the host larvae. Appl. Ent. Zool. 21(1): 114-118.
- Takabayashi, J. & S. Takahashi. 1989. Effects of host fecal pellet and synthetic kairomone on host-searching and postoviposition behavior of Apanteles kariyai, a parasitoid of Pseudaletia separata. Entomol. Exp. Appl. 52: 221-227.
- Takabayashi, J., N. Takashi & S. Takahashi. 1985. Effect of kairomones in the host searching behavior of Apanteles kariyai Watanabe (Hymenoptera: Braconidae), a parasitoid of the common armyworm, Pseudaletia separata Walker (Lepidoptera: Noctuidae). I. Presence of arresting stimulants produced by the host larvae. Appl. Ent. Zool. 20(4): 484-489.
- Takagi, M. 1975. An experimental study on the parasitism of Tetrastichus hagenowii (Ratzeburg) on the oothecae of Periplaneta fuliginosa (Serville). Jpn. J. Sanit. Zool. 26(2/3): 169-170.
- Takahashi, S., M. Hajika, J. Takabayashi & M. Fukui. 1990. Oviposition stimulants in the coccoid cuticular waxes of Aphytis yanonensis DeBach & Rosen. J. Chem. Ecol. 16(5): 1657-1665.
- Takahashi, S. & T. Sugai. 1982. Mating behavior of the parasitoid wasp Tetrastichus hagenowii (Hymenoptera: Eulophidae). Entomol. Gen. 7(4): 287-293.
- Thomson, M. S. & R. E. Stinner. 1988. Comparative responses of feral and laboratory Trichogramma spp. (Hymenoptera: Trichogrammatidae) to Heliothis spp. (Lepidoptera: Noctuidae) moth scales and inert particles. J. Entomol. Sci. 23(3): 245-250.
- Thomson, M. S. & R. E. Stinner. 1990. The scale response of Trichogramma (Hymenoptera: Trichogrammatidae): Variation among species in host specificity and the effect of conditioning. Entomophaga 35(1): 7-21.
- Turlings, T. C. J., J. H. Tumlinson, F. J. Eller & W. J. Lewis. 1991a. Larval-damaged plants: source of volatile synomones that guide the parasitoid Cotesia marginiventris to the micro-habitat of its host. Entomol. Exp. Appl. 58: 75-82.

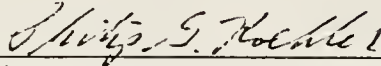
- Turlings, T. C. J., J. H. Tumlinson, R. R. Heath, A. T. Proveaux & R. E. Doolittle. 1991b. Isolation and identification of allelochemicals that attract the larval parasitoid, Cotesia marginiventris (Cresson), to the microhabitat of one of its hosts. *J. Chem. Ecol.* 17(11): 2235-2251.
- Turlings, T. C. J., J. H. Tumlinson & W. J. Lewis. 1990. Exploitation of herbivore-induced plant odors by host-seeking parasitic wasps. *Science* 250: 1251-1253.
- Van Driesche, R. G. & C. Hulbert. 1984. Host acceptance and discrimination by Comperia merceti (Compere) (Hymenoptera: Encyrtidae) and evidence for an optimal density range for resource utilization. *J. Chem. Ecol.* 10(9): 1399-1409.
- Van Leerdam, M. B., J. W. Smith, Jr. & T. W. Fuchs. 1985. Frass mediated, host-finding behavior of Cotesia flavipes, a braconid parasite of Diatraea saccharalis (Lepidoptera: Pyralidae). *Ann. Entomol. Soc. Am.* 78: 647-650.
- Vander Meer, R. K., F. Alvarez & C. S. Lofgren. 1988. Isolation of the trail recruitment pheromone of Solenopsis invicta. *J. Chem. Ecol.* 14(3): 825-838.
- Vargas, M. & F. Fallas. 1974. Notes on the biology of Tetrastichus hagenowii (Hymenoptera, Eulophidae) a parasite of cockroach oothecae. *Ent. News.* 85: 23-26.
- Vinson, S. B. 1975. Source of material in the tobacco budworm which initiates host-searching by the egg-larval parasitoid, Chelonus texanus. *Ann. Entomol. Soc. Am.* 68(2): 381-384.
- Vinson, S. B. 1976. Host selection by insect parasitoids. *Ann. Rev. Entomol.* 21: 109-133.
- Vinson, S. B. 1984a. The behavior of parasitoids, pp. 417-469. In G. A. Kerkut & L. I. Gilbert [eds.], *Comprehensive insect physiology, biochemistry, and pharmacology*, vol. 9. Pergamon Press, New York, New York.
- Vinson, S. B. 1984b. How parasitoids locate their hosts: a case of insect espionage, pp. 325-348. In T. Lewis [ed.], *Insect Communication*, 12th Symposium of the Royal Entomological Society of London, 7-9 September 1983.
- Vinson, S. B., D. P. Harlan & W. G. Hart. 1978. Response of the parasitoid Microterys flavus to the brown soft scale and its honeydew. *Environ. Entomol.* 7(6): 874-878.

- Vinson, S. B., R. D. Henson & C. S. Barfield. 1976. Ovipositional behavior of Bracon mellitor Say (Hymenoptera: Braconidae), a parasitoid of the boll weevil (Anthonomus grandis Boh.) I. Isolation and identification of a synthetic releaser of ovipositor probing. J. Chem. Ecol. 2(4): 431-440.
- Vinson, S. B., R. L. Jones, P. E. Sonnet, B. A. Bierl & M. Beroza. 1975. Isolation, identification and synthesis of host-seeking stimulants for Cardiochiles nigriceps, a parasitoid of tobacco budworm. Entomol. Exp. Appl. 18: 443-450.
- Vinson, S. B. & G. L. Piper. 1986. Source and characterization of host recognition kairomones of Tetrastichus hagenowii, a parasitoid of cockroach eggs. Physiol. Entomol. 11: 459-468.
- Weseloh, R. M. 1981. Host location by parasitoids, pp. 79-95. In D. A. Nordlund, R. L. Jones & W. J. Lewis [eds.], Semiochemicals: Their Role in Pest Control. Wiley & Sons, New York, New York.
- Weseloh, R. M. 1987. Orientation behavior and effect of experience and laboratory rearing on responses of Cotesia melanoscela (Ratzeburg) (Hymenoptera: Braconidae) to Gypsy moth silk kairomone. J. Chem. Ecol. 13(6): 1493-1502.
- Zaborski, E., P. E. A. Teal & J. E. Laing. 1987. Kairomone-mediated host finding by spruce budworm egg parasite, Trichogramma minutum. J. Chem. Ecol. 13(1): 113-122.

BIOGRAPHICAL SKETCH

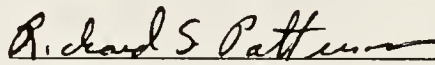
Daniel Robert Suiter was born on 6 April, 1963, in West Palm Beach, Florida, and is a sixth-generation native-born Floridian. Following graduation from Cardinal Newman High School in 1982, Dan entered Palm Beach Community College from which he received his Associate of Arts degree in 1985. In May 1987 he received his Bachelor of Science in Agriculture from the University of Florida, and Master of Science in urban entomology in 1989 under the direction of Drs. P. G. Koehler and R. S. Patterson. Dan is a member of the Entomological Society of America, the Florida Entomological Society, the International Society of Chemical Ecology, and Pi Chi Omega. During graduate studies, Dan performed a multitude of extension-type duties by providing training to the Florida Pest Control Association. He served as vice-president and then president of the University of Florida, Entomology & Nematology Student Organization in 1991-92 and 1992-93, respectively, and was the graduate student seminar series coordinator from 1990-92. He is engaged to be married to Ms. Lisa Ames of Marshfield, Mass. Dan and Lisa met in undergraduate school in 1986.

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree Doctor of Philosophy.



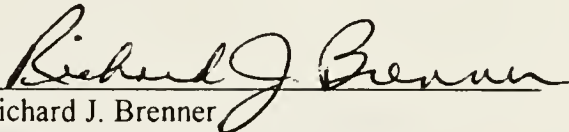
Philip G. Köehler, Chairman
Professor of Entomology and Nematology

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree Doctor of Philosophy.



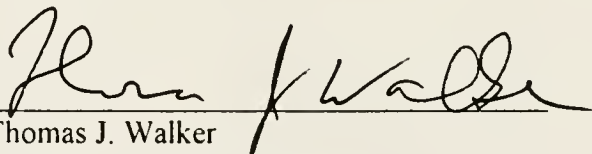
Richard S. Patterson
Professor of Entomology and Nematology

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree Doctor of Philosophy.



Richard J. Brenner
Assistant Professor of Entomology
and Nematology

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree Doctor of Philosophy.



Thomas J. Walker
Professor of Entomology and Nematology

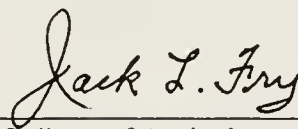
I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree Doctor of Philosophy.



Andre I. Khuri
Professor of Statistics

This dissertation was submitted to the Graduate Faculty of the College of Agriculture and to the Graduate School and was accepted as partial fulfillment of the requirements for the degree of Doctor of Philosophy.

August 1994



Dean, College of Agriculture

Dean, Graduate School

LD
1780
1994
.S948

UNIVERSITY OF FLORIDA



3 1262 08557 0660